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(54) Title: TREATMENT OF MAMMALIAN MYOCARDIUM WITH MORPHOGEN LOCALLY, OR WITH MORPHOGENICALLY-TREATED MYOGENIC PRECURSOR CELLS (57) Abstract The present invention provides methods for the treatment, and pharmaceuticals for use in the treatment, of mammalian subjects at risk of, or afflicted with, loss of or damage to myocardial tissue. The methods involve the administration of certain morphogens, inducers of those morphogens, agonists of the corresponding morphogen receptors, or small molecule morphogenic activators, or implantation of cells induced with those agents. The morphogens useful in the invention include OP1, CBMP-2A (BMP-2), CBMP-2B (BMP-4), and other members of the morphogens family of the TGF β superfamily of growth and differentiation factors.		

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**TREATMENT OF MAMMALIAN MYOCARDIUM WITH
MORPHOGEN LOCALLY, OR WITH MORPHOGENICALLY-TREATED
MYOGENIC PRECURSOR CELLS**

Field of the Invention

The present invention relates generally to methods and preparations for the treatment of mammals, including humans, at risk of, or afflicted with, loss of or damage to myocardium. The methods involve the implantation of mammalian myogenic precursor cells treated with certain morphogens, inducers of those morphogens, agonists of the corresponding morphogen receptors, or with small molecule morphogenic activators.

Background of the Invention

Unlike skeletal muscle or smooth muscle, adult mammalian cardiac muscle has extremely limited powers of growth and regeneration. During development, the myocardium arises by end-to-end fusion of myogenic precursor cells to form branched myofibers in which individual cardiac myocytes are joined by intercalated disks. The myogenic precursor cells which give rise to the myocardium are derived from the splanchnic mesoderm, which is derived from the lateral mesodermal mesenchyme which, in turn, arises from the mesoderm formed after gastrulation. It is generally believed that there are no remaining myogenic precursor cells in adult mammalian myocardium and, therefore, lost or damaged myocardium is typically replaced by fibrotic or scar tissue, rather than new myocardium. See, generally, B.M. Carlson, ed. (1981) Patten's Foundations of Embryology, 4th Edition, McGraw-Hill, New York. As a result, damage or loss of myocardium due, for example, to myocardial infarction, congestive heart failure, physical trauma (e.g., in an automobile accident), or infection, typically results in a permanent and often progressive loss of functional myocardium.

In contrast, mammalian skeletal muscle has much greater capacity for growth and regeneration, even in adulthood. Like the myocardium, skeletal muscle has its first origins after the induction of the mesoderm. After differentiation of the mesoderm into dorsal, intermediate, and lateral mesoderm, the dorsal mesodermal mesenchyme differentiates to form myotomes which, in turn, differentiate to form the myogenic precursor cells which ultimately form skeletal muscle. Unlike the myogenic precursor cells of the heart, the skeletal muscle precursors fuse side-to-side to form unbranched, multinucleated myofibers. Significantly, some portion of the

skeletal myogenic precursor cells do not differentiate into myocytes but, rather, attach to the plasmalemmas of the myocytes. These cells may remain, throughout adulthood, as largely undifferentiated, quiescent skeletal muscle "satellite cells." Upon injury of a skeletal muscle, however, these satellite cells are revealed to be myogenic precursor cells, or muscle "stem cells," which proliferate and differentiate into new and functional skeletal muscle. Even after injury, however, a portion of the proliferated satellite cells remain undifferentiated and attach to the newly formed myofibers. Thus, the satellite cells of skeletal muscle provide a constant and renewable source of myogenic precursor cells which allows for skeletal muscle repair and regeneration throughout mammalian life.

The proliferation and differentiation of skeletal muscle satellite cells has been extensively studied in vitro. For example, a simple saline extract of skeletal muscle has been shown to cause satellite cells to proliferate in culture (Bischoff (1989) in Myoblast Transfer Therapy, Griggs and Karpati, eds., pp. 147-158). Similarly, it has been shown that chick embryo extract or the conditioned medium of differentiated myotubes from young mice exhibits a strong mitogenic effect on satellite cells, but that conditioned medium from older murine myotubes has a lesser effect (Mezzogiorno et al. (1993) Mech. Ageing & Develop. 70:35-44). In addition, a number of hormones and growth factors have been found to enhance satellite cell proliferation, including FGF, PDGF, ACTH, LIF, and IGF (Bischoff (1989); Mezzogiorno et al. (1993)). Conversely, TGF- β_1 is widely believed to inhibit satellite cell proliferation, as does contact with the myofiber plasmalemma, but not the basal lamina (Bischoff (1989); but see Hathaway et al. (1991) J. Cell Physiol. 146:435-441).

Curiously, in a rat model of skeletal muscle injury, it was found that there were signs of satellite cell differentiation before there were significant signs of satellite cell proliferation (Rantanen et al. (1995) Lab. Invest. 72:341-347). This suggests the possibility that there are two populations of skeletal muscle satellite cells: "committed satellite cells" which respond to injury by rapidly differentiating to replace the injured tissue, and "stem satellite cells" which respond more slowly by proliferating and, perhaps, renewing the committed satellite cell population. In this scenario, the stem satellite cells may undergo mitosis to produce one daughter cell which remains a stem satellite cell, and another which becomes a committed satellite cell.

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In another animal model, autologous mouse skeletal muscle cells were explanted from a healthy muscle, proliferated in vitro, and then implanted into a necrotized skeletal muscle site (Alameddine and Fardeau (1989) in Myoblast Transfer Therapy, Griggs and Karpatti, eds., pp. 159-166). In these experiments, it was shown that the transplanted satellite cells were able to
5 populate the necrotized area and differentiate into functional myotubes. Similarly, PCT Publication WO 96/28541 discloses that histocompatible donor mouse myoblasts can be implanted into the weakened muscle of a mouse model of muscular dystrophy and differentiate into myofibers. In addition, it is shown that growth of the myoblasts in bFGF results in significantly more new myofibers at the implant site. Thus, skeletal muscle satellite cells,
10 proliferated in vitro, may be able to serve as a source of myogenic precursor cells for muscle restoration or regeneration therapy.

The ability of skeletal muscle satellite cells to restore or regenerate injured skeletal muscle, has led some researchers to test whether myogenic precursor cells could be used to replace lost or damaged myocardial muscle. For example, mouse fetal cardiomyocytes, which are not terminally
15 differentiated and retain the ability to divide, have been directly injected into the myocardium of a syngeneic adult mouse, and have been shown to form new and apparently functional myocardium (Soonpaa et al. (1994) Science 264:98-101). Significantly, it has been shown that skeletal muscle satellite cells, explanted from adult canine skeletal muscle can be proliferated in vitro and implanted into a site of myocardial cryoinjury, where they appear to differentiate into "cardiac-
20 like" muscle cells, possibly in response to morphogenic signals present in the myocardium (Chiu et al. (1995) Ann. Thorac. Surg. 60:12-18).

Morphogens and Growth Factors

A great many proteins have now been identified which appear to act as morphogenetic or growth factors, regulating cell proliferation and/or differentiation. Typically these growth factors
25 exert their effects on specific subsets of cells and/or tissues. Thus, for example, epidermal growth factors, nerve growth factors, fibroblast growth factors, various hormones, and many other proteins inducing or inhibiting cell proliferation or differentiation have been identified and shown to affect some subset of cells or tissues.

One group of morphogenetic proteins, referred to herein as "morphogens," includes
30 members of the family of bone morphogenetic proteins (BMPs) which were initially identified by

their ability to induce ectopic, endochondral bone morphogenesis. Subsequent characterization of the nucleic acid and amino acid sequences of the BMPs has shown them to be a subgroup of the TGF β superfamily of growth and differentiation factors. Members of the morphogen family have now been shown to include the mammalian osteogenic protein1 (OP1, also known as BMP7),
5 osteogenic protein2 (OP2), osteogenic protein3 (OP3), BMP2 (also known as BMP2A or CBMP2A), BMP3, BMP4 (also known as BMP2B or CBMP2B), BMP5, BMP6, Vgr1, and GDF1, as well as the Xenopus homologue Vgl and the Drosophila homologues DPP and 60A. Members of this family encode secreted polypeptides that share common structural features, and that are similarly processed from a pro-protein to yield a carboxy terminal mature protein of
10 approximately 100-110 amino acids. All members share a conserved pattern of cysteines in this domain and the active form of these proteins is either a disulfide-bonded homodimer of a single family member, or a heterodimer of two different members (see, e.g., Massague (1990) Annu. Rev. Cell Biol. 6:597; Sampath, et al. (1990) J. Biol. Chem. 265:13198).

The members of the morphogen family of proteins are expressed naturally in a variety of
15 tissues during development. BMP-2 (i.e., BMP-2A), for example, is expressed in embryonic mouse hair follicles, cartilage and bone (Lyons et al. (1989) Genes & Develop. 3:1657-1668); BMP3 has been shown to be most highly expressed in human embryonic lung and kidney, highly expressed in intestinal mucosa and skeletal tissues such as the perichondrium and periosteum, expressed in brain, but undetectable in embryonic heart and liver (Vukicevic et al. (1994) J. Histochem. Cytochem. 42:869-875); BMP4 has been shown to be expressed in the developing
20 limbs, heart, facial processes and condensed mesenchyme associated with early whisker follicles in embryonic mice (Jones, et al. (1991) Development 111:531-542); and OP1 (BMP7) has been shown immunohistochemically to be present in human embryos in sclerotome, hypertrophied chondrocytes, osteoblasts, periosteum, adrenal cortex, renal convoluted tubules, placenta,
25 smooth, cardiac and skeletal muscles, meninges and neural cells, as well as the basement membranes of the lungs, pancreas and skin (Vukicevic, et al. (1994) Biochem. Biophys. Res. Commun. 198:693-700). Some of the morphogens (e.g., OP2 and BMP2) were not detected in analyses of adult tissues, suggesting only an early developmental role for these morphogens (Ozkaynak, et al. (1992) J. Biol. Chem. 267:25220-25227).

Although, as noted above, several morphogens have been shown to be expressed in embryonic or adult mammalian heart tissue, and various utilities for the morphogens have been proposed and developed, it has never previously been shown or suggested that treatment of myogenic precursor cells with the morphogens, morphogen inducers, agonists of morphogen receptors, or small molecule morphogenic activators is useful in promoting the proliferation and/or differentiation of myogenic precursor cells into new and functional myocardium in a morphogenically permissive environment. Nor has it previously been shown or suggested that morphogenically-treated myogenic precursor cells are useful in the treatment of lost or damaged mammalian myocardium.

Summary of the Invention

The present invention is directed to methods of treatment, and pharmaceutical preparations for use in the treatment, of mammalian subjects at risk of, or afflicted with, loss of or damage to myocardium. Such subjects include subjects already afflicted with the loss of myocardial tissue, such as those which have already suffered a myocardial infarction, physical trauma to the heart (e.g., in an automobile accident, or those already suffering from congestive heart failure, as well as subjects reasonably expected to suffer from myocardial infarction or congestive heart failure. Whether a particular subject is at risk is a determination which may routinely be made by one of ordinary skill in the relevant medical or veterinary art.

In these methods of treatment, myogenic precursor cells are implanted into a mammal at a site at risk of, or afflicted with, loss of or damage to myocardium, and the myogenic precursor cells are morphogenically-treated prior to, simultaneously with, or subject to implantation. Thus, for example, morphogenically-treated mammalian myogenic precursor cells may be implanted into a mammalian heart at the site of a myocardial infarct, or into the damaged or weakened myocardium of a subject with congestive heart failure. The mammalian myogenic precursor cells may be derived from skeletal muscle (e.g., skeletal muscle satellite cells), from embryonic tissue (e.g., embryonic mesodermal mesenchyme) or from a myogenic precursor cell line maintained in vitro. Thus, the myogenic precursor cells may be derived from a donor (e.g., a tissue-type matched donor, sibling, identical twin, or fetus), may be derived from a tissue culture (e.g., undifferentiated or partly undifferentiated myogenic cells in culture; fetal tissue culture), or may be explanted from the subject and re-implanted after morphogen-induced proliferation and/or

differentiation. Finally, the morphogenic treatment of the implanted cells may include treatment of the cells with a morphogen, morphogen inducer, agonist of a morphogen receptor, or small molecule morphogenic activator prior to implantation, simultaneously with implantation, or subsequent to implantation.

5 The present invention is further directed to methods of promoting the proliferation and differentiation of mammalian myogenic precursor cells in vivo or in vitro. Thus, for example, myogenic precursor cells isolated from mammalian skeletal muscle tissue, embryonic myogenic precursor cells, or myogenic precursor cell lines, may be stimulated to proliferate by treatment with a morphogen, an inducer of a morphogen, an agonist of a morphogen receptor, or a small
10 molecule morphogenic activator. Alternatively, or in addition, mammalian myogenic precursor cells may be stimulated to differentiate into myocytes, particularly myocytes which express markers of myocardial tissue, in a morphogenically permissive environment.

 The present invention is further directed to therapeutic preparations comprising isolated mammalian myogenic precursor cells and an amount of a morphogen, inducer of a morphogen,
15 agonist of a morphogen receptor, or small molecule morphogenic activator sufficient to promote proliferation or differentiation of the myogenic precursor cells in a morphogenically permissive environment.

 The methods and compositions of the present invention capitalize in part upon the fact that certain proteins of eukaryotic origin, defined herein as morphogens, may be used to treat
20 myogenic precursor cells such that, when these morphogenically-treated myogenic precursor cells are present in a morphogenically permissive environment, they may migrate, proliferate and/or differentiate so as to form new and functional myocardium. In particular, the present invention is based in part upon the fact that treatment of myogenic precursor cells with these morphogens enhances or increases the probability, rate, or efficiency with which these cells migrate, proliferate
25 and/or differentiate into new and functional myocardium in a morphogenically permissive environment. Thus, in accordance with the present invention, morphogenically-treated myogenic precursor cells may be used to restore or regenerate lost or damaged myocardium in a mammal, or to prophylactically treat a mammal at risk of such loss or damage. The present invention is novel in that myocardial tissue is believed to lack a sufficient number of myogenic precursor cells
30 for adequate regeneration or repair of lost or damaged tissue and, therefore, the ability of the

morphogens to promote the migration, proliferation and/or differentiation of myogenic precursor cells (e.g., skeletal muscle satellite cells) into functional myocardium is unexpected.

In preferred embodiments, the morphogen is a dimeric protein comprising a pair of folded polypeptides, each having an amino acid sequence that shares a defined relationship with an amino acid sequence of a reference morphogen. Preferred morphogen polypeptides share a defined relationship with a sequence present in morphogenically active human OP-1 (SEQ ID NO: 4). However, any one or more of the naturally occurring or biosynthetic sequences disclosed herein similarly could be used as a reference sequence. Preferred morphogen polypeptides share a defined relationship with at least the C-terminal six cysteine domain of human OP-1 (residues 43-139 of SEQ ID NO: 4). Preferably, morphogen polypeptides share a defined relationship with at least the C-terminal seven cysteine domain of human OP-1 (residues 38-139 of SEQ ID NO: 4). That is, preferred morphogen polypeptides in a dimeric protein with morphogenic activity each comprise a sequence that corresponds to a reference sequence or is functionally equivalent thereto. Examples of preferred morphogens include mammalian, and particularly human, OP-1, CBMP-2A (BMP-2) and CBMP-2B (BMP-4).

Functionally equivalent sequences include functionally equivalent arrangements of cysteine residues disposed within the reference sequence, including amino acid insertions or deletions which alter the linear arrangement of these cysteines, but do not materially impair their relationship in the folded structure of the dimeric morphogen protein, including their ability to form such intra- or inter-chain disulfide bonds as may be necessary for morphogenic activity. Functionally equivalent sequences further include those wherein one or more amino acid residues differs from the corresponding residue of a reference morphogen sequence, e.g., the C-terminal seven cysteine domain (also referred to herein as the conserved seven cysteine skeleton) of human OP-1, provided that this difference does not destroy morphogenic activity. Accordingly, conservative substitutions of corresponding amino acids in the reference sequence are preferred. Amino acid residues that are "conservative substitutions" for corresponding residues in a reference sequence are those that are physically or functionally similar to the corresponding reference residues, e.g., that have similar size, shape, electric charge, chemical properties including the ability to form covalent or hydrogen bonds, or the like. Particularly preferred conservative substitutions are those fulfilling the criteria defined for an "accepted point mutation"

in Dayhoff, et al. (1978) Atlas of Protein Sequence and Structure, 5: Suppl. 3, ch. 22 (pp. 354-352), Natl. Biomed. Res. Found., Washington, D.C. 20007, the teachings of which are incorporated by reference herein.

In certain embodiments, a polypeptide suspected of being functionally equivalent to a reference morphogen polypeptide is aligned therewith using the method of Needleman, et al. (1970) J. Mol. Biol. 48:443-453, implemented conveniently by computer programs such as the Align program (DNASTar, Inc.). As noted above, internal gaps and amino acid insertions in the candidate sequence are ignored for purposes of calculating the defined relationship, conventionally expressed as a level of amino acid sequence homology or identity, between the candidate and reference sequences. "Amino acid sequence homology" is understood herein to include both amino acid sequence identity and similarity. Homologous sequences share identical and/or similar amino acid residues, where similar residues are conservative substitutions for, or "allowed point mutations" of, corresponding amino acid residues in an aligned reference sequence. Thus, a candidate polypeptide sequence that shares 70% amino acid homology with a reference sequence is one in which any 70% of the aligned residues are either identical to, or are conservative substitutions of, the corresponding residues in a reference sequence.

The present invention alternatively can be practiced with methods and compositions comprising a morphogen inducer in lieu of a morphogen. A "morphogen inducer" is a compound that stimulates the production (i.e., transcription, translation, and/or secretion) of morphogen by a cell competent to produce and/or secrete a morphogen encoded within the genome of the cell. Endogenous or administered morphogens can act as endocrine, paracrine or autocrine factors. Therefore, an inducer of a morphogen may stimulate endogenous morphogen synthesis by the cells in which the morphogenetic responses are induced, by neighboring cells in vivo or in vitro (e.g., in tissue culture) or by cells of a distant tissue in vivo (in which case the secreted morphogen is transported to the site of morphogenesis, e.g., by the individual's bloodstream). In preferred embodiments, the inducer stimulates expression and/or secretion of a morphogen so as to increase amounts thereof available to mammalian myogenic precursor cells in vivo or in vitro. Thus, to promote the migration, proliferation and/or differentiation of myogenic precursor cells in vivo, an inducer of a morphogen may be administered to induce production of morphogen by the myogenic precursor cells themselves, or by other cells co-cultured with the myogenic precursor

cells. Similarly, to promote the proliferation and/or differentiation of myogenic precursor cells in vivo, an inducer of a morphogen may administered locally or systemically to induce morphogen production by the myogenic precursor cells themselves, or by neighboring or distant cells in a mammal's body.

5 In still other embodiments, an agent which acts as an agonist of a morphogen receptor may be administered instead of the morphogen itself. An "agonist" of a receptor is a compound which binds to the receptor, and for which the result of such binding is similar to the result of binding the natural, endogenous ligand of the receptor. That is, the compound must, upon
10 interaction with the receptor, produce the same or substantially similar transmembrane and/or intracellular effects as the endogenous ligand. Thus, an agonist of a morphogen receptor binds to the receptor and such binding has the same or a functionally similar result as morphogen binding (e.g., induction of morphogenesis). The activity or potency of an agonist can be less than that of the natural ligand, in which case the agonist is said to be a "partial agonist," or it can be equal to or greater than that of the natural ligand, in which case it is said to be a "full agonist." Thus, for
15 example, a small peptide or other molecule which can mimic the activity of a morphogen in binding to and activating the morphogen's receptor may be employed as an equivalent of the morphogen. Preferably the agonist is a full agonist, but partial morphogen receptor agonists may also be advantageously employed. Methods of identifying such agonists are known in the art and include assays for compounds which induce morphogen-mediated responses (e.g., induction of
20 differentiation of metanephric mesenchyme, induction of endochondral bone formation, and the like). Such an agonist may also be referred to as a morphogen "mimic," "mimetic," or "analog."

Alternatively, a small molecule morphogenic activator, as described herein, may be administered instead of the morphogen itself to promote the migration, proliferation, and/or differentiation of myogenic precursor cells by increasing the level of expression of proteins
25 associated with myocardial phenotype. Exemplary methods comprise introducing a small molecule morphogenic activator that regulates some portion or portions of a morphogen-induced regulatory pathway, resulting in an effective increase in expression or activity of myocardium-specific protein. This may result either from stimulating an increase in the endogenous expression of such protein or from a decrease in the inhibition of normal expression of such protein. For
30 example, a small molecule morphogenic activator may act at the type I or type II morphogen

receptor; or at the serine/threonine kinase, or other kinase domains of those receptors. Another target of pathway activation is the Smad proteins, including the monomeric, dimeric (including heteromeric and homomeric complexes) or trimeric forms (including heteromeric and homomeric complexes). Alternately, a small molecule morphogenic activator may lead to activation of a transcription factor (for example, the X-protein shown in Figure 2) that causes phenotype-specific gene expression (i.e., expression of protein characteristic of myocardium).

Preferably, the morphogens, morphogen inducers, agonists of morphogen receptors, or small molecule morphogenic activators are directly contacted with the myogenic precursor cells in solution either in vitro prior to implantation, in vivo at the time of implantation, or in vivo subsequent to implantation. Alternatively, however, the morphogens, morphogen inducers, agonists of morphogen receptors may be administered by any route which is compatible with the selected agent, and may be formulated with any pharmaceutically acceptable carrier appropriate to the route of administration. Preferred systemic routes of administration are parenteral and, in particular, intravenous and intraperitoneal.

In additional embodiments, the present invention provides pharmaceutical compositions comprising a morphogen, or morphogen inducer, agonist of a morphogen receptor, or small molecule morphogenic activator in combination with one or more of a "muscle extract," conditioned medium from differentiated myotubes grown in culture, bFGF, IGF, PDGF, LIF, ACTH, MSH, or G-CSF. These compositions are useful in promoting the proliferation and/or differentiation of myogenic precursor cells.

Brief Description of the Figures

Figure 1. Panels 1-1 through 1-12 of this figure are a tabular alignment of the amino acid sequences of various naturally occurring morphogens with a preferred reference sequence of human OP1, residues 38-139 of SEQ ID NO: 4. Morphogen polypeptides shown in this figure also are identified in the Sequence Listing.

Figure 2. Figure 2 is a schematic representation of a morphogen-activated regulatory pathway for expression of a phenotype-specific gene.

Detailed Description of the Invention

I. Definitions

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In order to more clearly and concisely point out the subject matter of the claimed invention, the following definitions are provided for specific terms used in the following written description and appended claims.

Subjects at risk of, or afflicted with, loss of or damage to myocardium. As used herein, a
5 subject (preferably a mammal, e.g., a human) is said to be at risk of, or afflicted with, loss of or
damage to myocardium, if the subject has suffered a loss of functional myocardial tissue which is
clinically detectable in terms of reduced or altered cardiac function, or if the subject may
reasonably be expected to suffer such a loss. Subjects at risk of, or afflicted with, loss of or
damage to myocardium include, but are not limited to, subjects which have already suffered a
10 myocardial infarction, which have suffered a physical trauma to the heart (e.g., in an automobile
accident) which has reduced cardiac function, or which have already been diagnosed with
congestive heart failure; as well as subjects which can reasonably be expected to suffer a
myocardial infarction or congestive heart failure. Whether a particular subject is at risk is a
determination which may routinely be made by one of ordinary skill in the relevant medical or
15 veterinary art.

Myogenic precursor cells. As used herein, the term "myogenic precursor cells" refers to
cells capable of myogenesis, or the process of proliferation and differentiation into new and
functional muscle when present in a morphogenically permissive environment. Myogenic
precursor cells are variously referred to in the literature as "myoblasts," "muscle stem cells" or
20 "satellite cells."

Morphogenically permissive environment. As used herein, a "morphogenically permissive
environment" is an environment which allows or promotes the differentiation of cells into a
specific cell type or types. A "morphogenically permissive environment" is, therefore, sufficiently
free of inhibitors of cell differentiation to allow or promote cell differentiation. In addition, a
25 morphogenically permissive environment is one which provides signals (e.g., through cell-cell
contact, cell-extracellular matrix contact, or diffusible factors) which allow or promote a
pluripotent cell to follow a particular morphogenic pathway. In particular, with respect to
myocardial differentiation, a morphogenically permissive environment includes an environment of
intact or damaged myocardial tissue which provides signals to myogenic precursor cells which
30 allow or promote the differentiation of those cells into new and functional myocardium. It is

known, for example, that myogenic precursor cells differentiate into myocytes at least partly in response to contact with the plasmalemma of a myofiber. The presence of myofiber plasmalemmas, therefore, may be one element of a morphogenically permissive environment for myogenesis. Similarly, electrical or biochemical stimuli from nerves, as well as a variety of growth factors (see below), appear to be elements of a morphogenically permissive environment for myogenesis. Thus, a morphogenically permissive environment may include one or more of these elements.

II. Description of the Preferred Embodiments

A. General

The present invention depends, in part, upon the surprising discovery that morphogenically-treated mammalian myogenic precursors cells, when implanted in vivo at a site of lost or damaged mammalian myocardium, undergo a process of proliferation and/or differentiation to produce new and functional mammalian myocardium, thereby restoring or regenerating the lost or damaged tissue in whole or in part. This result is particularly unexpected in light of the fact that mammalian myocardial tissue is believed to lack a sufficient number of myogenic precursor cells for adequate regeneration or repair of lost or damaged tissue and, therefore, mammalian myocardium previously has been believed to be a poor responder for functional restoration or regeneration after tissue loss or damage. In addition, the present invention depends, in part, upon the surprising discovery that non-myocardial cells, such as those obtained from mammalian skeletal muscle or embryonic myogenic precursor cells, may be induced to proliferate and differentiate into myocardium in a morphogenically permissive environment. It is further surprising that the morphogens, morphogen inducers, agonists of morphogen receptors, and small molecule morphogenic activators, as described herein, may promote such restoration or regeneration despite the fact that they have no known role in myocardial tissue restoration or regeneration in the adult mammal.

Without being bound to any particular theory of the invention, it is believed that the morphogens, morphogen inducers, agonists of morphogen receptors, or small molecule morphogenic activators may promote the proliferation of myogenic precursor cells and render them more susceptible to differentiation into new and functional myocardium when implanted in a morphogenically permissive environment. Thus, it is believed that the morphogens, morphogen

inducers, agonists of morphogen receptors, or small molecule morphogenic activators may increase the pluripotentiality of these myogenic precursor cells, such that they may "switch fates" and, rather than differentiating only into smooth or skeletal muscle, they may proliferate and then differentiate into new and functional myocardium.

5 B. Isolating and Culturing Mammalian Myogenic Precursor Cells

Methods of isolating and culturing mammalian myogenic precursor cells are well-established in the art. For example, myogenic precursor cells may be obtained, as further described in the examples below, by dissociation of skeletal muscle and subsequent culturing of the satellite cells. Alternatively, myogenic precursor cells may be obtained from embryonic
10 tissues, where they arise as fetal myoblasts from the myotomes of the somites, after induction of the mesoderm. Myogenic precursor cells may also be obtained from cell lines, such as a pluripotent mesodermal mesenchyme cell line or a partially dedifferentiated laboratory cell line, which may be induced to differentiate into myoblasts after implantation into a morphogenically permissive environment. See, generally, Hathaway, et al. (1991) J. Cell. Physiol. 146:435-441;
15 Mezzogiorno et al. (1993) Mech. Ageing & Develop. 70:35-44; Alameddine and Fardeau (1989); Chiu et al. (1995) Ann. Thorac. Surg. 60:12-18.

1. Isolating Myogenic Precursor Cells from Skeletal Muscle

In preferred embodiments, the myogenic precursor cells are obtained from skeletal muscle. The skeletal muscle donor is preferably the subject for myocardial treatment or an identical twin in
20 order to avoid problems of histocompatibility and possible tissue rejection. Alternatively, other family members or histocompatible donors, including transgenic mammals raised for organ transplantation purposes (e.g., lacking MHC markers or expressing humanized MHC proteins), may be employed as donors of the skeletal muscle tissue. Depending upon the degree of histocompatibility, standard methods of immunosuppression may be needed in conjunction with
25 the present invention to prevent rejection of the implanted cells.

Briefly, a sample of skeletal muscle is excised from one or more skeletal muscles of a subject under local or general anesthesia. Any excessive connective tissue and fasciae are dissected away, the muscle is rinsed in sterile solution, and the muscle is dissociated by, for example, mincing with scissors or passage through a meat grinder until substantially
30 homogeneous. The amount of muscle excised will depend, of course, upon the quantity of

myogenic precursor cells required by the treatment, as well as the degree of myogenic precursor cell proliferation which is to be promoted in vitro. Typically, however, amounts of 1-100 grams, more preferably 10-50 grams, of skeletal muscle tissue are removed. Such quantities may be excised conveniently from one or more of the larger, relatively superficial muscles of the limbs (e.g., biceps brachii, triceps brachii, brachialis, brachioradialis, rectus femoris, biceps femoris, semitendinosus, gracilis, vastus lateralis, gastrocnemius, tibialis anterior), chest and shoulders (e.g., pectoralis, deltoid), pelvis and hips (e.g., gluteus medius, gluteus maximus), back (e.g., trapezius, latissimus dorsi) or abdomen (e.g., obliquus abdominis externus, rectus abdominis), but may be obtained from any available skeletal muscle.

Preferably, the dissociated muscle then is incubated with a proteolytic enzyme (e.g., pronase (Sigma, St. Louis, MO), collagenase (Sigma, St. Louis, MO), hyaluronidase (Sigma, St. Louis, MO), or trypsin (Difco Laboratories, Inc., Detroit, MI) at 37°C for 15 min to 1 hr to remove remaining connective tissue. The mass of digested muscle tissue optionally may be further dissociated by, for example, repeated pipetting or mixing. In addition, the digested mass optionally may be washed, pelleted and resuspended to remove digested connective tissue and enzyme, and any remaining debris may be removed by filtration. The cells are then suspended in a sterile buffer (e.g., phosphate buffered saline solution) and centrifuged at approximately 500-550 g for approximately 10 minutes to sediment the larger, multinucleated skeletal muscle fibers and myocytes, while leaving the satellite cells in the supernatant. Either before or after centrifugation, serum, such as fetal bovine serum (FBS, GIBCO BRL, Grand Island, NY), may be added to the mixture to halt the enzymatic cleavage process and antibiotics may be added to prevent microbial growth. If desired, satellite cells may be separated from fibroblasts and other remaining cells using a density centrifugation method (see, e.g., Yablonka-Reuveni and Nameroff (1987) Histochemistry 87:27-38).

2. Isolating Myogenic Precursor Cells from Embryos

Myogenic precursors cells may be isolated from mammalian embryonic or fetal (together "embryonic") tissues at various stages of development after induction of the mesoderm. Thus, for example, myogenic precursor cells may be obtained from the embryonic mesoderm prior to its further differentiation into dorsal, intermediate, and lateral mesodermal mesenchyme. After this stage of differentiation, any mesodermal cells may be employed but, preferably, cells are employed

which arise along the routes of differentiation toward skeletal or cardiac muscle. For example, the dorsal mesodermal mesenchyme differentiates to form the myotomes which, in turn, differentiate to form both the skeletal muscles of the trunk and the limb buds. The mesodermal mesenchyme of the limb buds further differentiates to form the skeletal muscles of the appendages (as well as the appendicular skeleton. Similarly, the lateral mesodermal mesenchyme differentiates, in part, to form the splanchnic mesoderm which, in turn, differentiates to form the myocardium and smooth muscles of the viscera (as well as the gonads, circulatory system and other primary elements of the viscera). One of ordinary skill in the art may, therefore, readily choose appropriate embryonic cells for use in the present invention (see, e.g., Soonpaa et al. (1994) Science 264:98-101; also see, generally, B.M. Carlson, ed. (1981) Patten's Foundations of Embryology, 4th Edition, McGraw-Hill, New York). Once excised, the embryonic tissue may be treated essentially as described above with respect to skeletal muscle to isolate the myogenic precursor cells.

As with cells obtained from the skeletal muscle of an adult mammal, histocompatibility problems may arise upon implantation of embryonic myogenic precursor cells. Therefore, depending upon the degree of histocompatibility, standard methods of immunosuppression may be needed in conjunction with the present invention to prevent rejection of the implanted cells.

3. Isolating Myogenic Precursor Cells from Established Cell Lines

Established cell lines, including myogenic precursor cell lines, myoblast cell lines, or mesenchymal cell lines, may also be employed in the present invention without the need for isolation of the myogenic precursor cells from adult or embryonic tissue. For example, the established murine myoblast cell line C₂C₁₂ (ATCC CRL 1772) has been implanted into mouse hearts and shown to differentiate into functional myocardium and fuse with native myocardium (Koh et al. (1993) J. Clin. Invest. 92:1548-54). Alternatively, pluripotent mesodermal stem cell lines, including primary dermal fibroblast lines, smooth muscle cell lines, or chondroblast lineages may be caused to differentiate into muscle cells (see, e.g., Choi et al. (1990) Proc. Nat. Acad. Sci. (USA) 87:7988-7992). Finally, it should be noted that a variety of established mammalian myogenic cell lines are commercially available for use in accordance with the present invention including, for example, the human cell line HISM (ATCC CRL 1692), the murine cell lines C2C12 (ATCC CRL 1772), NOR-10 (ATCC CRL 197), and G-8 (ATCC CRL 1456), and the rat

cell lines A7r5 (ATCC CRL 1444), A10 (ATCC CRL 1476), H9c2 (2-1) (ATCC CRL 1446), L6 (ATCC CRL 1458) and L8 (ATCC CRL 1769). Following essentially the same protocols as described in the original reports of these cell lines (see the ATCC's Catalogue of Cell Lines & Hybridomas, for citations) one of ordinary skill in the art can readily produce comparable cell lines from any mammalian species.

4. Culturing Myogenic Precursor Cells

Myogenic precursor cells may be cultured on solid or in liquid media. Thus, for example, the myogenic precursor cells may be suspended in a flask of liquid medium while maintaining mild or periodic agitation. Alternatively, the cells may be plated on a solid substrate and fed with a liquid medium. Appropriate liquid media are well known in the art and include, but are not limited to, McCoy's, M199, Minimal Essential Medium (MEM), Dulbecco's Modified Eagle Medium (commercially available from, for example, GIBCO BRL, Grand Island, NY, or Sigma Chemical Company, St. Louis, MO), and the like. These media may, of course, be supplemented with additional buffers or nutrient solutions (e.g., 10% fetal bovine serum, 3% horse serum), or with antimycotics and/or antibiotics (e.g., 50-5,000 IU/ml penicillin, 50-5,000 µg/ml streptomycin, 5-50 µg/ml gentamicin). Preferably, the liquid media is replaced every 24-48 hrs and the cultures are maintained at a relatively constant temperature of about 37°C under a normal or 5% CO₂-enriched humid atmosphere. For culturing on solid substrates, cells are preferably plated at a density of approximately 10⁴-10⁶ cells per 60 mm plate. To promote cell adherence to solid substrates, the plates may optionally be coated with, for example, basement membrane matrigel or laminin (Sigma Chemical Company, St. Louis, MO) although, as described below, adherence and/or confluence may inhibit proliferation.

In order to allow or promote proliferation of the myogenic precursor cells in vitro while inhibiting premature differentiation, a number of steps may be taken. For example, myogenic precursor cell proliferation has been shown to be inhibited by TGF-β (Allen and Boxhorn (1989) J. Cell Physiol. 138:311-315) and contact with myofiber plasmalemmas, (Bischoff (1989)); and has been shown to be promoted by a saline "muscle extract" (Bischoff (1986) Dev. Biol. 115:140), conditioned medium from differentiated myotubes grown in culture (Mezzogiorno et al. (1993) Mech. Ageing & Develop. 70:35-44), basic fibroblast growth factor (bFGF) (Clegg et al. (1987) J. Cell. Biol. 105:949-56), insulin-like growth factors (IGF) (Ewton and Florini (1977)

Endocrinology 106:577-587; Tollfsen et al. (1989) Proc. Nat. Acad. Sci. (USA) 1543-1547), platelet-derived growth factor (PDGF) (Yablonka-Reuveni et al. (1990) J. Cell Biol. 111:1623-1629), leukemia-inhibiting factor (LIF) (Austin and Burgess (1991) J. Neuro. Sci. 101:193-197), adrenocorticotrophic hormone (ACTH) (Cossu et al. (1989) Develop. Biol. 131:331-336; De Angelis et al. (1992) Dev. Biol. 151:446-458), melanocyte-stimulating factor (MSH) (Cossu et al. (1989) Develop. Biol. 131:331-336) and granulocyte colony stimulating factor (G-CSF) (Austin and Burgess (1991) J. Neuro. Sci. 101:193-197). Thus, in order to promote proliferation of the myogenic precursor cells in vitro prior to implantation and/or in vivo after implantation, the cells may be grown in the presence of one or more of these factors, or other known mitogens. In addition, as is generally known in the art, proliferation of such cells may be promoted by repeated passaging (e.g., treatment with dilute trypsin to remove adhered cells from the culture plate and replating at a lower density every 2-3 days), growth in liquid culture, growth in the absence of enhancers of cell adhesion, growth in the presence of inhibitors of cell adhesion, and/or growth at densities below confluence.

There is no absolute requirement that the myogenic precursor cells of the present invention be cultured in vitro prior to implantation. Indeed, if a therapeutically effective number of myogenic precursor cells can conveniently and economically be obtained without culturing, this step may be omitted. On the other hand, when such cells are in scarce supply (e.g., from fetal tissues) or can be obtained only through invasive measures (e.g., excision of substantial portions of muscle from a donor or donor/subject), it is preferred that smaller numbers of cells be obtained initially, and then proliferated in vitro. Doubling times will vary depending upon the source of cells, media, and the presence or absence of other growth factors, but doubling times on the order of every 12 hrs have been reported in the literature for muscle satellite cells grown in the presence of muscle abstract (Bischoff, (1989)). Therefore, it is contemplated that culturing times of several days to a week may be employed in the present methods to expand the myogenic precursor cell population prior to implantation.

Myogenic precursor cells may be harvested by brief trypsin treatment to remove any cells adhered to the culture plate or vessel, and centrifugation (e.g., 10-15 min at 500-1000 g). The cells may then be resuspended in a physiologically acceptable buffer solution (e.g., PBS, Ringer's saline) at an appropriate density (e.g., 10^3 - 10^7 cells/ml).

Finally, it should be noted that morphogens, morphogen inducers, agonists of morphogen receptors, and small molecule morphogenic activators may be used to treat the myogenic precursor cells during culturing (if any) to aid in proliferation and/or subsequent differentiation. Alternatively, the myogenic precursor cells may be treated with a morphogen, morphogen inducer, agonist of a morphogen receptor, or small molecule morphogenic activator either simultaneously with, or subsequent to, implantation. In the case of morphogen inducers, the myogenic precursor cells may be co-cultured with auxiliary cells which respond to these morphogen inducers by producing morphogen. The myogenic precursor cells then may be implanted along with these auxiliary cells, or may be isolated from the co-culture by standard cell separation techniques, which are known in the art, but which will vary with the type of auxiliary cells employed (e.g., density centrifugation separation, cell type specific cytotoxins).

C. Implantation of Myogenic Precursor Cells at a Myocardial Site

Myogenic precursor cells may be implanted at a site of loss of or damage to mammalian myocardium by any of a variety of surgical techniques known in the art. These techniques range from the minimally invasive (e.g., injection by needle through the thoracic wall) to substantially invasive (e.g., thoracotomy and incision of the myocardium, followed by implantation, suturing of the implant site and closing of the chest). The technique employed in any given instance will depend upon such factors as the size of the myocardial site to be treated, the accessibility of the site, and the age and stamina of the subject.

Generally, the myogenic precursor cells are implanted in a physiologically acceptable buffer solution. To minimize the volume of solution administered to the treatment site, the cells may be at a relatively high titer within this solution (e.g., 10^5 - 10^7 cells/ml). The solution may contain growth factors, as described above, to promote further proliferation of the myogenic precursor cells within the implant site, or may be free of such factors so as to promote differentiation into new and functional myocardium in the morphogenically permissive environment of the myocardial implant site. In addition, as noted above, the myogenic precursor cells may be implanted either simultaneously with a morphogen, morphogen inducer, agonist of a morphogen receptor, or small molecule morphogenic activator, or the morphogenic treatment may be subsequent to implantation.

Thus, for example, a solution of myogenic precursor cells and a morphogen, morphogen inducer, agonist of a morphogen receptor, or small molecule morphogenic activator, may be implanted at a site of myocardial infarction in essentially the following manner. For example, to treat a myocardial infarct to the anterior wall of the left ventricle, a left thoracotomy is performed on a subject under general anesthesia in an intercostal space (e.g., the sixth intercostal space) and the site of the infarct is determined by observation. At the discretion of the surgeon, the heart may or may not be stopped and systemic blood flow shunted to a heart-lung machine. Myogenic precursor cells then may be directly injected into one or more sites within the infarct using an intravenous catheter (e.g., a 16-gauge Teflon catheter from Criticon, Tampa, FL). The initial injection(s) may include a morphogen, morphogen inducer, agonist of a morphogen receptor, or small molecule morphogenic activator, or these may be included in one or more additional injections to the infarct site. Alternatively, a number of non-transmural incisions may be made at the site of the infarct to create "channels" parallel to the direction of the myocardial fibers. The suspension of myogenic precursor cells (with or without morphogen, morphogen inducer or morphogen receptor agonist) then may be introduced within these channels and the channels closed by suturing. Finally, the pericardium is sutured and chest wall are closed by standard surgical techniques (after restarting and returning systemic circulation to the heart from a heart-lung machine, if employed).

The treatment of chronically deteriorating mammalian myocardium (e.g., due to congestive heart failure or chronic myopathy), may be performed similarly except that the implantation sites are chosen to correspond to areas of generalized myocardial deterioration and, therefore, may be more diffuse.

The number of myogenic precursor cells implanted will vary according to the amount of myocardial tissue to be restored or regenerated. The volume of cells to be restored or regenerated may be ascertained by standard techniques of cardiac imaging. Generally, it is expected that on the order of approximately 10^4 - 10^5 myogenic precursor cells will be required to restore or regenerate 1 mg of myocardial tissue (see, e.g., Alameddine and Fardeau (1989)).

D. Morphogens, Inducers, Agonists, and Small Molecule Morphogenic Activators

Morphogens useful in the present invention include eukaryotic proteins originally identified as osteogenic proteins (see U.S. Patent 5,011,691, incorporated herein by reference).

such as the OP1, OP2, OP3, CBMP2A (BMP-2), CBMP-2B (BMP-4) and BMP3 proteins (SEQ ID NOs: 4-9, 15-22, 25-27), as well as amino acid sequence-related proteins such as DPP (SEQ ID NO: 10, from Drosophila), Vgl (SEQ ID NO: 11, from Xenopus), Vgr1 (SEQ ID NO: 12, from mouse), GDF1 (SEQ ID NOs: 13, 30 and 31, from humans, see Lee (1991), PNAS 88:4250-4254), 60A (SEQ ID NOs: 23 and 24, from Drosophila, see Wharton et al. (1991) PNAS 88:9214-9218), dorsalin-1 (from chick, see Basler et al. (1993) Cell 73:687-702 and GenBank accession number L12032) and GDF5 (from mouse, see Storm et al. (1994) Nature 368:639-643). Additional useful morphogens include biosynthetic morphogen constructs disclosed in U.S. Pat. No. 5,011,691, e.g., COP1, 3-5, 7 and 16, as well as others known in the art including dor3, NODAL, UNIVIN, BMP9, BMP10, GDF3, GDF6, GDF7, CDMP2, and SCREW. See also U.S. Pat. No. 4,968,590, incorporated herein by reference.

Naturally occurring proteins identified and/or appreciated herein to be morphogens form a distinct subgroup within the loose evolutionary grouping of sequence-related proteins known as the TGF β superfamily or supergene family. The naturally occurring morphogens share substantial amino acid sequence homology in their C-terminal regions (domains). Typically, the above-mentioned naturally occurring morphogens are translated as a precursor, having an N-terminal signal peptide sequence, typically less than about 30 residues, followed by a "pro" domain that is cleaved to yield the mature C-terminal domain. The signal peptide is cleaved rapidly upon translation, at a cleavage site that can be predicted in a given sequence using the method of Von Heijne (1986) Nucleic Acids Research 14:4683-4691. The pro domain typically is about three times larger than the fully processed mature C-terminal domain. Herein, the "pro" form of a morphogen refers to a morphogen comprising a folded pair of polypeptides each comprising the pro and mature domains of a morphogen polypeptide. Typically, the pro form of a morphogen is more soluble than the mature form under physiological conditions. The pro form appears to be the primary form secreted from cultured mammalian host cells.

Table 1, below, summarizes various naturally occurring morphogens identified to date, including their nomenclature as used herein, their Sequence Listing references, and publication sources for the amino acid sequences for the full length proteins not included in the Sequence Listing. Each of the generic terms set forth in Table 1 is intended and should be understood to embrace morphogenically active proteins expressed from nucleic acids encoding the identified

sequence mentioned below and set forth in the Sequence Listing, or a morphogenically active fragment or precursor thereof, including functional equivalents such as naturally occurring and biosynthetic variants thereof. Naturally occurring variants include allelic variant forms isolated from other individuals of a single biological species, and phylogenetic counterpart (species) variant forms (homologues) isolated from phylogenetically distinct biological species. The disclosures of publications mentioned below are incorporated herein by reference.

TABLE 1

10	"OP1"	Refers generically to morphogenically active proteins expressed from nucleic acids encoding OP1 proteins, including at least the human OP1 protein disclosed in SEQ ID NO: 4 ("hOP1"), and the mouse OP1 protein disclosed in SEQ ID NO: 5 ("mOP1"). In each of human and mouse OP1 proteins, the conserved seven cysteine skeleton is defined by residues 38 to 139. cDNA sequences and amino acid sequences encoded therein and corresponding to the full length proteins are provided in SEQ ID NOs: 15 and 16 (hOP1) and SEQ ID NOs: 17 and 18 (mOP1.) The mature proteins are defined by residues 293-431 (hOP1) and 292-430 (mOP1). The "pro" regions of the proteins, cleaved to yield the mature, morphogenically active proteins are defined essentially by residues 30-292 (hOP1) and residues 30-291 (mOP1).
15		
20	"OP2"	Refers generically to morphogenically active proteins expressed from nucleic acids encoding the OP2 proteins, including at least the human OP2 protein disclosed in SEQ ID NO: 6 ("hOP2"), and the mouse OP2 protein disclosed in SEQ ID NO: 7 ("mOP2"). In each of human and mouse OP2 proteins, the conserved seven cysteine skeleton is defined by residues 38 to 139 of SEQ ID NOs: 6 and 7. cDNA sequences and amino acid sequences encoded therein and corresponding to the full length proteins are provided in SEQ ID NOs: 19 and 20 (hOP2) and SEQ ID NOs: 21 and 22 (mOP2.) The mature proteins are defined essentially by residues 264-402 (hOP2) and 261-399 (mOP2). The "pro" regions of the proteins, cleaved to yield the mature, morphogenically active proteins are defined essentially by residues 18-263 (hOP2) and residues 18-260 (mOP1).
25		

"OP3" Refers generically to morphogenically active proteins expressed from nucleic acids encoding OP3 proteins, including at least the mouse OP3 protein disclosed in SEQ ID NO: 26 ("mOP3"). The conserved seven cysteine domain is defined by residues 298 to 399 of SEQ ID NO: 26, which shares greater than 79% amino acid identity with the corresponding mOP2 and hOP2 sequences, and greater than 66% identity with the corresponding OP1 sequences. A cDNA sequence encoding the above-mentioned amino acid sequence is provided in SEQ ID NO: 25. OP3 is unique among the morphogens identified to date in that the residue at position 9 in the conserved seven cysteine domain (e.g., residue 315 of SEQ ID NO: 26) is a serine, whereas other morphogens typically have a tryptophan at this location.

"CBMP2" Refers generically to morphogenically active proteins expressed from nucleic acids encoding the CBMP2 proteins, including at least the human CBMP2A protein disclosed in SEQ ID NO: 8 (hCBMP2A) and the human CBMP2B protein disclosed in SEQ ID NO: 9 (hCBMP2B). The amino acid sequence for the full length proteins, referred to in the literature as BMP2A and BMP2B, or BMP2 and BMP4, appear in Wozney, et al. (1988) Science 242:1528-1534. The pro domain for BMP2 (BMP2A) likely includes residues 25-248 of the published sequence; the mature protein, residues 249-396. The pro domain for BMP4 (BMP2B) likely includes residues 25-256 of the published sequence; the mature protein, residues 257-408.

"DPP" Refers generically to proteins encoded by the Drosophila DPP gene and defining at least the conserved seven cysteine skeleton of SEQ ID NO: 10. The amino acid sequence for the full length protein appears in Padgett, et al. (1987) Nature 325:81-84. The pro domain likely extends from the signal peptide cleavage site to residue 456 of the published sequence; the mature protein likely is defined by residues 457-588.

"Vgl" Refers generically to proteins encoded by the Xenopus Vgl gene and defining at least the conserved seven cysteine skeleton of SEQ ID NO: 11. The amino acid sequence for the full length protein appears in Weeks (1987) Cell 51:861-867. The

prodomain likely extends from the signal peptide cleavage site to residue 246 of the published sequence; the mature protein likely is defined by residues 247-360.

"Vgr1"

Refers generically to proteins encoded by the murine Vgr1 gene and defining at least the conserved seven cysteine skeleton of SEQ ID NO: 12. The amino acid sequence for the full length protein appears in Lyons, et al. (1989) PNAS 86:4554-4558. The prodomain likely extends from the signal peptide cleavage site to residue 299 of the published sequence; the mature protein likely is defined by residues 300-438.

"GDF1"

Refers generically to proteins encoded by the human GDF1 gene and defining at least the conserved seven cysteine skeleton of SEQ ID NO: 13. The cDNA and encoded amino sequence for the full length protein are provided in SEQ ID NOs: 30 and 31. The prodomain likely extends from the signal peptide cleavage site to residue 214; the mature protein likely is defined by residues 215-372.

"60A"

Refers generically to morphogenically active proteins expressed from nucleic acid encoding 60A proteins or morphogenically active fragments thereof, including at least the Drosophila 60A protein disclosed in SEQ ID NO: 24. A Drosophila 60A cDNA is disclosed in SEQ ID NO: 23. The prodomain likely extends from the signal peptide cleavage site to residue 324; the mature protein likely is defined by residues 325-455. The active fragment of 60A protein likely is defined by the conserved seven cysteine skeleton of residues 354 to 455 of SEQ ID NO: 24. The 60A protein is considered likely herein to be a phylogenetic counterpart variant of the human and mouse OP1 genes; Sampath, et al. (1993) PNAS 90:6004-6008.

"BMP3"

Refers generically to proteins encoded by the human BMP3 gene and defining at least the conserved seven cysteine skeleton of SEQ ID NO: 27. The amino acid sequence for the full length protein appears in Wozney, et al. (1988) Science 242:1528-1534. The pro domain likely extends from the signal peptide cleavage

site to residue 290 of the published sequence; the mature protein likely is defined by residues 291-472.

5 "BMP5" Refers generically to proteins encoded by the human BMP5 gene and defining at least the conserved seven cysteine skeleton of SEQ ID NO: 28. The amino acid sequence for the full length protein appears in Celeste, et al. (1991) PNAS 87:9843-9847. The pro domain likely extends from the signal peptide cleavage site to residue 316 of the published sequence; the mature protein likely is defined by residues 317-454.

10 "BMP6" Refers generically to proteins encoded by the human BMP6 gene and defining at least the conserved seven cysteine skeleton of SEQ ID NO: 29. The amino acid sequence for the full length protein appears in Celeste, et al. (1990) PNAS 87:9843-5847. The pro domain likely extends from the signal peptide cleavage site to residue 374 of the published sequence; the mature protein likely is defined by residues 375-513.

15 As shown in Figure 1, the OP2 and OP3 proteins have an additional cysteine residue in the conserved C-terminal region (e.g., see residue 41 of SEQ ID NOs: 6 and 7), in addition to the conserved cysteine skeleton or domain in common with the other known proteins in this family. The GDF1 protein has a four amino acid insert within the conserved skeleton (residues 44-47 of SEQ ID NO: 13) but this insert likely does not interfere with the relationship of the cysteines in
20 the folded structure. Further, the CBMP2 proteins are missing one amino acid residue within the cysteine skeleton. Thus, these morphogen polypeptides illustrate the principles of alignment used herein with respect to the preferred reference morphogen sequence of human OP1, residues 38-139 of SEQ ID NO: 4.

25 In certain preferred embodiments, morphogens useful herein include those in which the amino acid sequences of morphogen polypeptides comprise a sequence sharing at least 70% amino acid sequence homology or "similarity", and preferably 80% homology or similarity with a reference morphogen sequence selected from the foregoing sequences or naturally occurring morphogens. Preferably, the reference morphogen is human OP1, and the reference sequence thereof is the C-terminal seven cysteine domain present in morphogenically active forms of human

OP1, residues 38-139 of SEQ ID NO: 4. Morphogens useful herein accordingly include alleles, phylogenetic counterparts and other variants of the preferred reference sequence, whether naturally-occurring or biosynthetically produced (e.g., including "muteins" or "mutant proteins"), as well as novel members of the morphogenic family of proteins including the morphogens set forth and identified above, e.g., in connection with Table 1. Certain particularly preferred morphogen polypeptides share at least 60% amino acid identity with the preferred reference sequence of human OP1, still more preferably at least 65% amino acid identity therewith.

In other preferred embodiments, the family of morphogen polypeptides useful in the present invention, and members thereof, are defined by a generic amino acid sequence. For example, Generic Sequence 7 (SEQ ID NO: 1) and Generic Sequence 8 (SEQ ID NO: 2) disclosed below, accommodate the homologies shared among preferred morphogen protein family members identified to date, including at least OP1, OP2, OP3, CBMP2A, CBMP2B, BMP3, BMP5, BMP6, DPP, Vg1, Vgr1, 60A, and GDF1. The amino acid sequences for these proteins are described herein (see Sequence Listing) and/or in the art, as summarized above. The generic sequences include both the amino acid identity shared by these sequences in the C-terminal domain, defined by the six and seven cysteine skeletons (Generic Sequences 7 and 8, respectively), as well as alternative residues for the variable positions within the sequence. The generic sequences provide an appropriate cysteine skeleton where inter- or intramolecular disulfide bonds can form, and contain certain critical amino acids likely to influence the tertiary structure of the folded proteins. In addition, the generic sequences allow for an additional cysteine at position 41 (Generic Sequence 7) or position 46 (Generic Sequence 8), thereby encompassing the morphogenically active sequences of OP2 and OP3.

Generic Sequence 7 (SEQ ID NO: 1)

			Leu	Xaa	Xaa	Xaa	Phe	Xaa	Xaa
			1				5		
Xaa	Gly	Trp	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Pro
		10					15		
Xaa	Xaa	Xaa	Xaa	Ala	Xaa	Tyr	Cys	Xaa	Gly
		20					25		
Xaa	Cys	Xaa	Xaa	Pro	Xaa	Xaa	Xaa	Xaa	Xaa
		30					35		
Xaa	Xaa	Xaa	Asn	His	Ala	Xaa	Xaa	Xaa	Xaa
		40					45		

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Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa
		50					55		
Xaa	Xaa	Xaa	Cys	Cys	Xaa	Pro	Xaa	Xaa	Xaa
		60					65		
Xaa	Xaa	Xaa	Xaa	Xaa	Leu	Xaa	Xaa	Xaa	Xaa
		70					75		
Xaa	Xaa	Xaa	Val	Xaa	Leu	Xaa	Xaa	Xaa	Xaa
		80					85		
Xaa	Met	Xaa	Val	Xaa	Xaa	Cys	Xaa	Cys	Xaa
		90					95		

wherein each Xaa independently is selected from a group of one or more specified amino acids defined as follows: "res." means "residue" and Xaa at res. 2 = (Tyr or Lys); Xaa at res. 3 = Val or Ile; Xaa at res. 4 = (Ser, Asp or Glu); Xaa at res. 6 = (Arg, Gln, Ser, Lys or Ala); Xaa at res. 7 = (Asp or Glu); Xaa at res. 8 = (Leu, Val or Ile); Xaa at res. 11 = (Gln, Leu, Asp, His, Asn or Ser); Xaa at res. 12 = (Asp, Arg, Asn or Glu); Xaa at res. 13 = (Trp or Ser); Xaa at res. 14 = (Ile or Val); Xaa at res. 15 = (Ile or Val); Xaa at res. 16 = (Ala or Ser); Xaa at res. 18 = (Glu, Gln, Leu, Lys, Pro or Arg); Xaa at res. 19 = (Gly or Ser); Xaa at res. 20 = (Tyr or Phe); Xaa at res. 21 = (Ala, Ser, Asp, Met, His, Gln, Leu or Gly); Xaa at res. 23 = (Tyr, Asn or Phe); Xaa at res. 26 = (Glu, His, Tyr, Asp, Gln, Ala or Ser); Xaa at res. 28 = (Glu, Lys, Asp, Gln or Ala); Xaa at res. 30 = (Ala, Ser, Pro, Gln, Ile or Asn); Xaa at res. 31 = (Phe, Leu or Tyr); Xaa at res. 33 = (Leu, Val or Met); Xaa at res. 34 = (Asn, Asp, Ala, Thr or Pro); Xaa at res. 35 = (Ser, Asp, Glu, Leu, Ala or Lys); Xaa at res. 36 = (Tyr, Cys, His, Ser or Ile); Xaa at res. 37 = (Met, Phe, Gly or Leu); Xaa at res. 38 = (Asn, Ser or Lys); Xaa at res. 39 = (Ala, Ser, Gly or Pro); Xaa at res. 40 = (Thr, Leu or Ser); Xaa at res. 44 = (Ile, Val or Thr); Xaa at res. 45 = (Val, Leu, Met or Ile); Xaa at res. 46 = (Gln or Arg); Xaa at res. 47 = (Thr, Ala or Ser); Xaa at res. 48 = (Leu or Ile); Xaa at res. 49 = (Val or Met); Xaa at res. 50 = (His, Asn or Arg); Xaa at res. 51 = (Phe, Leu, Asn, Ser, Ala or Val); Xaa at res. 52 = (Ile, Met, Asn, Ala, Val, Gly or Leu); Xaa at res. 53 = (Asn, Lys, Ala, Glu, Gly or Phe); Xaa at res. 54 = (Pro, Ser or Val); Xaa at res. 55 = (Glu, Asp, Asn, Gly, Val, Pro or Lys); Xaa at res. 56 = (Thr, Ala, Val, Lys, Asp, Tyr, Ser, Gly, Ile or His); Xaa at res. 57 = (Val, Ala or Ile); Xaa at res. 58 = (Pro or Asp); Xaa at res. 59 = (Lys, Leu or Glu); Xaa at res. 60 = (Pro, Val or Ala); Xaa at res. 63 = (Ala or Val); Xaa at res. 65 = (Thr, Ala or Glu); Xaa at res. 66 = (Gln, Lys, Arg or Glu); Xaa at res. 67 = (Leu, Met or Val); Xaa at res. 68 =

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(Asn, Ser, Asp or Gly); Xaa at res. 69 = (Ala, Pro or Ser); Xaa at res. 70 = (Ile, Thr, Val or Leu); Xaa at res. 71 = (Ser, Ala or Pro); Xaa at res. 72 = (Val, Leu, Met or Ile); Xaa at res. 74 = (Tyr or Phe); Xaa at res. 75 = (Phe, Tyr, Leu or His); Xaa at res. 76 = (Asp, Asn or Leu); Xaa at res. 77 = (Asp, Glu, Asn, Arg or Ser); Xaa at res. 78 = (Ser, Gln, Asn, Tyr or Asp); Xaa at res. 79 = (Ser, Asn, Asp, Glu or Lys); Xaa at res. 80 = (Asn, Thr or Lys); Xaa at res. 82 = (Ile, Val or Asn); Xaa at res. 84 = (Lys or Arg); Xaa at res. 85 = (Lys, Asn, Gln, His, Arg or Val); Xaa at res. 86 = (Tyr, Glu or His); Xaa at res. 87 = (Arg, Gln, Glu or Pro); Xaa at res. 88 = (Asn, Glu, Trp or Asp); Xaa at res. 90 = (Val, Thr, Ala or Ile); Xaa at res. 92 = (Arg, Lys, Val, Asp, Gln or Glu); Xaa at res. 93 = (Ala, Gly, Glu or Ser); Xaa at res. 95 = (Gly or Ala) and Xaa at res. 97 = (His or Arg).

Generic Sequence 8 (SEQ ID NO: 2) includes all of Generic Sequence 7 and in addition includes the following sequence (SEQ ID NO: 14) at its N-terminus:

Cys	Xaa	Xaa	Xaa	Xaa
1				5

15

Accordingly, beginning with residue 7, each "Xaa" in Generic Sequence 8 is a specified amino acid defined as for Generic Sequence 7, with the distinction that each residue number described for Generic Sequence 7 is shifted by five in Generic Sequence 8. Thus, "Xaa at res. 2 = (Tyr or Lys)" in Generic Sequence 7 refers to Xaa at res. 7 in Generic Sequence 8. In Generic Sequence 8, Xaa at res. 2 = (Lys, Arg, Ala or Gln); Xaa at res. 3 = (Lys, Arg or Met); Xaa at res. 4 = (His, Arg or Gln); and Xaa at res. 5 = (Glu, Ser, His, Gly, Arg, Pro, Thr, or Tyr).

As noted above, certain currently preferred morphogen polypeptide sequences useful in this invention have greater than 60% identity, preferably greater than 65% identity, with the amino acid sequence defining the conserved six or seven cysteine skeleton of hOP1 (e.g., residues 43-139 or 38-139 of SEQ ID NO: 4). These particularly preferred sequences include allelic and phylogenetic counterpart variants of the OP1 and OP2 proteins, including the *Drosophila* 60A protein (SEQ ID NO: 24). Accordingly, in certain particularly preferred embodiments, useful morphogens include active proteins comprising pairs of polypeptide chains within the generic

amino acid sequence herein referred to as "OPX" (SEQ ID NO: 3), which corresponds to the seven cysteine skeleton and accommodates the homologies between several identified variants of OP1 and OP2. As described therein, each Xaa at a given position independently is selected from the residues occurring at the corresponding position in the C-terminal sequence of mouse or human OP1 or OP2 (see SEQ ID NOs: 4-7 and/or SEQ ID NOs: 15-22).

In still other preferred embodiments, useful morphogen polypeptides have amino acid sequences comprising a sequence encoded by a nucleic acid that hybridizes, under stringent hybridization conditions, to DNA or RNA encoding reference morphogen sequences, e.g., C-terminal sequences defining the conserved seven cysteine domains of OP1 or OP2, e.g., nucleotides 1036-1341 and nucleotides 1390-1695 of SEQ ID NO: 15 and 19, respectively. As used herein, stringent hybridization conditions are defined as hybridization according to known techniques in 40% formamide, 5 X SSPE, 5 X Denhardt's Solution, and 0.1% SDS at 37°C overnight, and washing in 0.1 X SSPE, 0.1% SDS at 50°C.

As noted above, morphogens useful in the present invention generally are dimeric proteins comprising a folded pair of the above polypeptides. Morphogens are inactive when reduced, but are active as oxidized homodimers and when oxidized in combination with other morphogens of this invention to produce heterodimers. Thus, members of a folded pair of morphogen polypeptides in a morphogenically active protein can be selected independently from any of the specific morphogen polypeptides mentioned above.

The morphogens useful in the methods, compositions and devices of this invention include proteins comprising any of the polypeptide chains described above, whether isolated from naturally-occurring sources, or produced by recombinant DNA or other synthetic techniques, and includes allelic and phylogenetic counterpart variants of these proteins, as well as biosynthetic variants (muteins) thereof, and various truncated and fusion constructs. Deletion or addition mutants also are envisioned to be active, including those which may alter the conserved C-terminal six or seven cysteine domain, provided that the alteration does not functionally disrupt the relationship of these cysteines in the folded, biologically active, structure. Accordingly, such active forms are considered the equivalent of the specifically described constructs disclosed herein. The proteins may include forms having varying glycosylation patterns, varying N-termini, a family of related proteins having regions of amino acid sequence homology, and active truncated

or mutated forms of native or biosynthetic proteins, produced by expression of recombinant DNA in host cells.

The morphogenic proteins can be expressed from intact or truncated cDNA or from synthetic DNAs in prokaryotic or eukaryotic host cells, and purified, cleaved, refolded, and
5 dimerized to form morphogenically active compositions. Currently preferred host cells include E. coli or mammalian cells, such as CHO, COS or BSC cells. A detailed description of the morphogens useful in the methods, compositions and devices of this invention is disclosed in published application WO92/15323, the disclosure of which is incorporated by reference herein.

Thus, in view of this disclosure, skilled genetic engineers can isolate genes from cDNA or
10 genomic libraries of various different biological species, which encode appropriate amino acid sequences, or construct DNAs from oligonucleotides, and then can express them in various types of host cells, including both prokaryotes and eukaryotes, to produce large quantities of active proteins capable of stimulating the morphogenesis of, and/or inhibiting damage or loss of, mammalian myocardial tissue.

15 As noted above, a protein is morphogenic herein generally if it induces the developmental cascade of cellular and molecular events that culminate in the formation of new, organ-specific tissue. Preferably, a morphogen comprises a pair of polypeptides having a sequence that corresponds to or is functionally equivalent to at least the conserved C-terminal six or seven cysteine skeleton of human OP1, included in SEQ ID NO: 4. The morphogens generally are
20 competent to induce a cascade of events including all of the following, in a morphogenically permissive environment: stimulating proliferation of progenitor cells; stimulating the differentiation of progenitor cells; stimulating the proliferation of differentiated cells; and supporting the growth and maintenance of differentiated cells. Details of how the morphogens useful in this invention first were identified, as well as a description on how to make, use and test
25 them for morphogenic activity are disclosed in published application WO92/15323. As disclosed therein, the morphogens can be purified from naturally-sourced material or recombinantly produced from prokaryotic or eukaryotic host cells, using the genetic sequences disclosed therein. Alternatively, novel morphogenic sequences can be identified following the procedures disclosed therein.

Exemplary useful morphogens include naturally derived proteins comprising a pair of polypeptides, the amino acid sequences of which comprise sequences selected from those disclosed in the Sequence Listing and Figure 1. Other useful sequences include those of the naturally derived morphogens dorsalin-1, SCREW, NODAL, UNIVIN and GDF5, discussed
5 herein in connection with Table 1, as well as biosynthetic constructs disclosed in U.S. Pat. 5,011,691, the disclosure of which is incorporated herein by reference (e.g., COP1, COP3, COP4, COP5, COP7, and COP16).

Accordingly, certain preferred morphogens useful in the methods and compositions of this invention can be described as morphogenically active proteins having amino acid sequences
10 sharing 70% or, preferably, 80% homology with a reference morphogen sequence described above, e.g., residues 38-139 of SEQ ID NO: 4, where "homology" is as defined herein above. Alternatively, in other preferred embodiments, morphogens useful in the methods and compositions disclosed herein fall within the family of polypeptides described by Generic Sequence 7, SEQ ID NO: 1, more preferably by Generic Sequence 8, SEQ ID NO: 2.

15 Figure 1 herein sets forth an alignment of the amino acid sequences of the active regions of exemplary naturally occurring proteins that have been identified or appreciated herein as morphogens, including human OP1 (hOP1, SEQ ID NOs: 4 and 15-16), mouse OP1 (mOP1, SEQ ID NOs: 5 and 17-18), human and mouse OP2 (SEQ ID NOs: 6, 7, and 19-22), mouse OP3 (SEQ ID NOs: 25-26), CBMP2A (SEQ ID NO: 8), CBMP2B (SEQ ID NO: 9), BMP3 (SEQ ID
20 NO: 27), DPP (from Drosophila, SEQ ID NO: 10), Vgl, (from Xenopus, SEQ ID NO: 11); Vgr1 (from mouse, SEQ ID NO: 12), GDF1 (from mouse and/or human, SEQ ID NOs: 13, 30 and 31), 60A protein (from Drosophila, SEQ ID NOs: 23 and 24), BMP5 (SEQ ID NO: 28) and BMP6 (SEQ ID NO: 29). The sequences are aligned essentially following the method of Needleman, et al. (1970) J. Mol. Biol., 48:443-453, calculated using the Align Program (DNASTar, Inc.). In
25 Figure 1, three dots indicates that the amino acid in that position is the same as the corresponding amino acid in hOP1. Three dashes indicates that no amino acid is present in that position, and are included for purposes of illustrating homologies. For example, amino acid residue 60 is "missing" in both CBMP2A and CBMP2B. Of course, both of these amino acid sequences in this region comprise Asn-Ser (residues 58, 59), with CBMP2A then comprising Lys and Ile, whereas CBMP-
30 2B comprises Ser and Ile. Figure 1 also illustrates the handling of insertions in the morphogen

amino acid sequence: between residues 56 and 57 of BMP3 is an inserted Val residue; between residues 43 and 44 of GDF1 is inserted the amino acid sequence, Gly-Gly-Pro-Pro. Such deviations from the reference morphogen sequence are ignored for purposes of calculating the defined relationship between, e.g., GDF1 and hOP1. As is apparent from the amino acid
5 sequence comparisons set forth in Figure 1, significant amino acid changes can be made from the reference sequence while retaining morphogenic activity. For example, while the GDF1 protein sequence depicted in Figure 1 shares only about 50% amino acid identity with the hOP1 sequence described therein, the GDF1 sequence shares greater than 70% amino acid sequence homology with the hOP1 sequence, where "homology" is as defined above.

10 In other embodiments, as an alternative to the administration of a morphogenic protein, an effective amount of an agent competent to stimulate or induce increased endogenous morphogen expression in a mammal may be administered by any of the routes described herein. Such an inducer of a morphogen may be provided to a mammal, e.g., by local or systemic administration to the mammal or by direct administration to implanted myogenic precursor cells, or may be
15 provided to auxiliary cells co-cultured with myogenic precursor cells. Methods for identifying and testing inducers (stimulating agents) competent to modulate the level of production of morphogens by a given tissue or cell type are described in detail in published applications WO93/05172 and WO93/05751, the teachings of which are incorporated herein by reference. Briefly, candidate compounds can be identified and tested by incubation in vitro with a test tissue
20 or cells thereof, or a cultured cell line derived therefrom, for a time sufficient to allow the compound to affect the production, i.e., the expression and/or secretion, of a morphogen produced by the cells of that tissue. Suitable tissue, or cultured cells of a suitable tissue, preferably can be selected from renal epithelium, ovarian tissue, fibroblasts, and osteoblasts.

In other embodiments, an agent which acts as an agonist of a morphogen receptor may be
25 administered instead of the morphogen itself. Such an agent may also be referred to as a morphogen "mimic," "mimetic," or "analog." Thus, for example, a small peptide or other molecule which can mimic the activity of a morphogen in binding to and activating the morphogen's receptor may be employed as an equivalent of the morphogen. Preferably the agonist is a full agonist, but partial morphogen receptor agonists may also be advantageously
30 employed. Methods of identifying such agonists are known in the art and include assays for

compounds which induce morphogen-mediated responses (e.g., induction of differentiation of metanephric mesenchyme, induction of endochondral bone formation). For example, methods of identifying morphogen inducers or agonists of morphogen receptors may be found in U.S. Ser. No. 08/478,097 filed June 7, 1995 and U.S. Ser. No. 08/507,598 filed July 26, 1995, the disclosures of which are incorporated herein by reference.

In yet other embodiments, a small molecule morphogenic activator may be used for promoting the migration, proliferation, and/or differentiation of myogenic precursor cells by increasing the level of expression of proteins associated with myocardial phenotype. Exemplary methods comprise introducing a small molecule morphogenic activator that regulates some portion or portions of a morphogen-induced regulatory pathway, resulting in an effective increase in expression or activity of myocardium-specific protein. This may result either from stimulating an increase in the endogenous expression of such protein or from a decrease in the inhibition of normal expression of such protein. For example, a small molecule morphogenic activator may act at the type I or type II morphogen receptor; or at the serine/threonine kinase, or other kinase domains of those receptors. Another target of pathway activation is the Smad proteins, including the monomeric, dimeric (including heteromeric and homomeric complexes) or trimeric forms (including heteromeric and homomeric complexes). The Smads have been characterized, and are known in the art. See, e.g., Baker, et al., Curr. Op. Genet. Develop., 7: 467-473 (1997), incorporated by reference herein.

Alternately, a small molecule morphogenic activator may lead to activation of a transcription factor (for example, the X-protein shown in Figure 2) that causes phenotype-specific gene expression (i.e., expression of protein characteristic of myocardium). A small molecule morphogenic activator may act to facilitate, mimic, or, if desired, prevent any one or several of the following: type I and/or type II receptor binding, phosphorylation of the type I receptor, phosphorylation of the Smad molecules, Smad complex formation, Smad translocation into the nucleus, nuclear accumulation of the Smad complex, or transcription modulation of the Smad complex. Furthermore, a small molecule morphogenic activator may act on Smads or Smad complexes to alter tertiary structure, thereby to facilitate or inhibit interaction of the Smad or Smad complex with a receptor kinase domain, other Smads, DNA binding proteins, or DNA itself.

In a particularly-preferred embodiment, a small molecule morphogenic activator is contacted with myogenic precursor cells in vivo or in vitro, or is administered to a patient, wherein the small molecule morphogenic activator facilitates formation of Smad complexes, particularly complexes comprising molecules of Smad1, Smad2, Smad3, Smad4, Smad5 and/or Smad8 in order to induce myogenic precursor cells to migrate, proliferate and/or differentiate into cells expressing markers of a myocardial tissue phenotype. Also in a preferred embodiment, methods comprise administering a small molecule morphogenic activator composition that activates a serine/threonine kinase domain associated with a morphogen type I or type II receptor, thereby to activate the pathway involved in morphogen-induced gene expression. In another embodiment, methods of the invention comprise activating Smad4 association with Smad1, thereby to induce morphogen-responsive phenotype. Methods of the invention may also facilitate Smad interaction with specific nucleic acids, such as promoters of myocardial tissue phenotype-specific gene expression (i.e., expression of genes for a phenotypic protein; a protein associated with preservation, restoration, or enhancement of phenotype, including a protein which is critical for production of non-protein phenotypic markers, such as characteristic lipids or carbohydrates; a protein associated with performance of a phenotypic function or morphology; or a morphogen). Such interaction may be, for example, in association with a transcription control factor that is capable of binding to a regulatory portion of a gene and, simultaneously, to one or more regulatory proteins such as a Smad complex (See Figure 2).

An exemplary morphogen-activated pathway is shown in Figure 2. Morphogens are ligands for the type I and type II receptors. Following phosphorylation of the type I receptor by the type-II receptor, the type I receptor specifically phosphorylates Smad1 homodimers. The type I receptor also specifically phosphorylates Smad5 homodimers. The homodimers then separate to form, in association with a phosphorylated Smad4 molecule, a phosphorylated heteromeric complex comprising at least a Smad1 and a Smad4. A phosphorylated Smad1/Smad5/Smad4 heterotrimer may alternatively be formed. The heteromeric complex then translocates into the nucleus, and accumulates therein. In the nucleus, the Smad complex binds operative DNA, either alone or in association with a specific DNA binding protein (the X-protein in Figure 2), to initiate DNA transcription. The "X-protein" acts as a DNA-binding protein, binding the Smad heteromeric complex to the DNA. The Smad1, Smad2, Smad3 and Smad5 proteins consist of

conserved amino- and carboxy-terminal domains linked by a region that is more divergent among the Smads. The carboxy-terminal domain has an effector function. The amino-terminal domain interacts physically with the carboxy-terminal domain, inhibiting its effector activity, and contributes to DNA binding. Receptor-mediated phosphorylation of the serine residues at the end
5 of the carboxy-terminal domain relieves the carboxy-terminal domain from the inhibitory action of the amino-terminal domain. Phosphorylated Smad molecules form a heteromeric complex with at least one other specific Smad family molecule. The resulting Smad complex then translocates into and accumulates in the cell nucleus. There, the heteromeric Smad complexes regulate transcriptional responses either alone or by specific interaction with a DNA-binding protein, such
10 as forkhead activin signal transducer-1 (FAST1).

Other intracellular pathways are induced by morphogens, and may be affected in the manner described herein by use of a small molecule morphogenic activator.

In a preferred embodiment, a small molecule morphogenic activator for use in the invention is a compound that affects one or more intracellular pathways that normally are under
15 morphogen regulation. Such small molecule morphogenic activators preferably have the ability to enter the cell and target one or more intracellular pathway components in order to stimulate or inhibit their activity. For example, a small molecule morphogenic activator that promotes Smad complex formation between Smad1, Smad4, and Smad5 will stimulate pathways leading to expression of genes encoding phenotype-specific proteins.

20 One way in which to identify a candidate small molecule morphogenic activator is to assay for the ability of the candidate to modulate the effective systemic or local concentration of a morphogen. This may be done, for example, by incubating the candidate in a cell culture that produces the morphogen, and assaying the culture for a parameter indicative of a change in the production level of the morphogen according the methods of U.S.S.N. 08/451,953 and/or U.S.
25 5,650,276, the teachings of each of which are incorporated by reference herein. Alternatively, candidate compounds are screened for their ability to induce phenotype-specific protein production in a cell culture in which morphogen activity is not present. Examples of compositions which may be screened for their effect on the production of morphogens or other phenotype-specific proteins include but are not limited to chemicals, biological response modifiers
30 (e.g., lymphokines, cytokines, hormones, or vitamins), plant extracts, microbial broths and

extracts medium conditioned by eukaryotic cells, body fluids, or tissue extracts. Useful candidate compositions then may be tested for in vivo efficacy in a suitable animal model. These compositions then may be used in vivo to up-regulate morphogen-activated regulatory pathways of phenotype-specific protein expression.

- 5 A simple method of determining if a small molecule composition has effected a change in the level of a phenotype-specific protein in cultured cells is provided in co-owned, co-pending patent application, U.S.S.N. 08/451,953, the disclosure of which is incorporated by reference herein. The level of a target phenotype-specific protein in a cell resulting from exposure to a small molecule is measured. Alternatively, a change in the activity or amount of an intracellular
- 10 pathway component is measured in response to application of a candidate small molecule. Candidates having the desired affect on protein production or pathway regulation are selected for use in methods of the invention. If, for example, a composition up-regulates the production of OP-1 by a kidney cell line, it would then be desirable to test systemic administration of this compound in an animal model to determine if it up-regulates the production of OP-1 in vivo.
- 15 The level of morphogen in the body may be a result of a wide range of physical conditions, e.g., tissue degeneration such as occurs in diseases including arthritis, emphysema, osteoporosis, kidney diseases, lung diseases, cardiomyopathy, and cirrhosis of the liver. The decrease in level of morphogens in the body may also occur as a result of the normal process of aging. The same strategy is used for compositions affecting intracellular pathway components. A composition
- 20 selected by these screening methods is then used as a treatment or prophylactic.

- An appropriate test cell is any cell comprising DNA defining a morphogen-responsive transcription activating element operatively associated with a reporter gene encoding a detectable phenotype-specific gene product. Such DNA can occur naturally in a test cell or can be a transfected DNA. The induced intracellular effect typically is characteristic of morphogenic
- 25 biological activity, such as Smad activation, or activation of a cascade of biochemical events, such as described above, or involving, for example, cyclic nucleotides, diacylglycerol, and/or and other indicators of intracellular signal transduction such as activation or suppression of gene expression, including induction of mRNA resulting from gene transcription and/or induction of protein synthesis resulting from translation of mRNA transcripts indicative of tissue morphogenesis.
- 30 Exemplary morphogen-responsive cells are preferably of mammalian origin and include, but are

not limited to, osteogenic progenitor cells; calvaria-derived cells; osteoblasts; osteoclasts; osteosarcoma cells and cells of hepatic or neural origin. Any such morphogen responsive cell can be a suitable test cell for assessing whether a candidate substance is a small molecule morphogenic activator.

5 A preferred identification method is carried out by exposing a test cell to at least one candidate substance, and detecting whether such exposure induces expression of the detectable phenotype-specific gene product that is in operative association with the morphogen-responsive transcription activating element. Expression of this gene product indicates that the candidate substance induces a morphogen-mediated biological effect. Skilled artisans can, in light of
10 guidance provided herein, construct a test cell with a responsive element from a morphogen-responsive cell and a reporter gene of choice, using recombinant vectors and transfection techniques well-known in the art. There are numerous well-known reporter genes useful herein. These include, for example, chloramphenicol acetyltransferase (CAT), luciferase, human growth hormone (hGH), beta-galactosidase, and assay systems and reagents which are available through
15 commercial sources. As will be appreciated by skilled artisans, the listed reporter genes represent only a few of the possible reporter genes that can be used herein. Examples of such reporter genes can be found in Ausubel et al., Eds., Current Protocols in Molecular Biology, John Wiley & Sons, New York, (1989). Broadly, any gene that encodes a detectable product, e.g., any product having detectable enzymatic activity or against which a specific antibody can be raised, can be
20 used as a reporter gene in the present identification method.

A currently preferred reporter gene system is the firefly luciferase reporter system. Gould, et al., Anal. Biochem., 7:404-408 (1988), incorporated herein by reference. The luciferase assay is fast and sensitive. In this assay system, a lysate of the test cell is prepared and combined with ATP and the substrate luciferin. The encoded enzyme luciferase catalyzes a rapid, ATP-
25 dependent oxidation of the substrate to generate a light-emitting product. The total light output is measured and is proportional to the amount of luciferase present over a wide range of enzyme concentrations. CAT is another frequently used reporter gene system; a major advantage of this system is that it has been extensively validated and is widely accepted as a measure of promoter activity. Gorman, et al., Mol. Cell. Biol., 2:1044-1051 (1982), incorporated by
30 reference herein. In this system, test cells are transfected with CAT expression vectors and

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incubated with the candidate substance within 2-3 days of the initial transfection. Thereafter, cell extracts are prepared. The extracts are incubated with acetyl CoA and radioactive chloramphenicol. Following the incubation, acetylated chloramphenicol is separated from nonacetylated form by thin layer chromatography. In this assay, the degree of acetylation reflects the CAT gene activity with the particular promoter.

Another suitable reporter gene system is based on immunologic detection of hGH. This system is also quick and easy to use. Selden, et al., Mol. Cell. Biol., 6:3173-3179 (1986), incorporated by reference herein. The hGH system is advantageous in that the expressed hGH polypeptide is assayed in the media, rather than in a cell extract. Thus, this system does not require the destruction of the test cells. It will be appreciated that the principle of this reporter gene system is not limited to hGH but rather adapted for use with any polypeptide for which an antibody of acceptable specificity is available or can be prepared.

A small molecule morphogenic activator composition may up-regulate a morphogen-activated pathway by acting at any one or more point. For example, small molecule morphogenic activator potentiation of the pathway may be initiated at the receptor level. Depending on the pathway, the transmembrane receptors may be type I and/or type II, or may be comprise variations on either type I or type II receptors. For example, OP-1 is capable of activating regulatory pathways comprising at least two variations of both type I and type II receptors (ActR-I and BMPR-1B, and ActR-II and BMPR-II, respectively). A small molecule morphogenic activator may stimulate the pathway by acting as a ligand and binding to any of the receptors, thereby inducing phosphorylation of type I receptors and/or Smad molecules. Similarly, a small molecule morphogenic activator may activate the regulatory pathway at the level of the serine/threonine kinase domain of the receptors, thereby stimulating phosphorylation of type I receptors and/or Smad molecules.

As a further alternative, a small molecule morphogenic activator may activate the regulatory pathway at the level of Smad complex formation. A small molecule morphogenic activator may stimulate the formation of Smad family homodimers, heterodimers, or other homomeric or heteromeric complexes. Furthermore, a small molecule morphogenic activator may activate the pathway by interacting with a Smad molecule or Smad complex, thereby altering its tertiary structure.

Alternatively, or in addition, a small molecule morphogenic activator may activate the regulatory pathway by facilitating translocation of a Smad molecule or Smad complex or accumulation of the Smad molecule or Smad complex within the nucleus of the cell. By acting as a DNA binding protein or a transcriptional activator, a small molecule morphogenic activator may activate the regulatory pathway by increasing transcriptional activity caused by the Smad molecule or Smad complex.

Furthermore, a small molecule morphogenic activator can act to stimulate the regulatory pathway by interfering with an inhibitor of the pathway. For example, Smad6 and Smad7, which are structurally different than Smad1, Smad2, Smad3 and Smad5, act as inhibitors of certain types of desirable phenotype-specific protein expression (e.g., by activating TGF- β to induce scar tissue formation). Smad6 forms a stable association with type I receptors and interferes with the phosphorylation of other Smad proteins, including Smad2 and Smad 1, and their subsequent heteromerization with Smad4. Smad7 also forms a stable association with activated type I receptors and blocks access and phosphorylation of certain Smad molecules, thereby preventing formation of certain Smad heteromeric complexes. Smad7 also inhibits nuclear accumulation of Smad heteromeric complexes. A small molecule morphogenic activator may interfere with the inhibitory activity of these Smad proteins by, for example, tightly binding to either one or both proteins and rendering either protein incapable of stable association with type I receptors, or by competitively binding and stimulating the morphogen-activated transmembrane receptors. Alternatively, a small molecule morphogenic activator may activate the inhibitory effects of these Smads in order to inhibit an undesirable effect (e.g., TGF β activity).

E. Subjects for Treatment

As a general matter, the methods of the present invention may be utilized for any mammalian subject at risk of, or afflicted with, loss of or damage to myocardium. Mammalian subjects which may be treated according to the methods of the invention include, but are not limited to, human subjects or patients. In addition, however, the invention may be employed in the treatment of domesticated mammals which are maintained as human companions (e.g., dogs, cats, horses), which have significant commercial value (e.g., dairy cows, beef cattle, sporting animals), which have significant scientific value (e.g., captive or free specimens of endangered species), or which otherwise have value. In addition, as a general matter, the subjects for

treatment with the methods of the present invention need not present indications for morphogen treatment other than those associated with loss of or damage to myocardium. That is, the subjects for treatment generally are expected to be otherwise free of indications for morphogen treatment. In some number of cases, however, the subjects may present with other symptoms
5 (e.g., osteoporosis, chronic renal failure) for which morphogen treatment also would be indicated. In such cases, the morphogen treatment should be adjusted accordingly to avoid excessive dosing.

One of ordinary skill in the medical or veterinary arts is trained to recognize subjects at risk of, or afflicted with, loss of or damage to myocardium. In particular, clinical and non-clinical indications, as well as accumulated experience, relating to the presently disclosed and other
10 methods of treatment, are expected to inform the skilled practitioner in deciding whether a given individual is a subject at risk of, or afflicted with, loss of or damage to myocardium and whether any particular treatment is best suited to the subject's needs, including treatment according to the present invention.

As a general matter, a mammalian subject may be regarded as a subject at risk of, or
15 afflicted with, loss of or damage to myocardium if that subject has already been diagnosed as at risk of, or afflicted with, loss of or damage to myocardium. Such subjects include, but are not limited to, those which have already suffered a myocardial infarction, which have suffered a physical trauma to the heart, or which have been diagnosed with congestive heart failure.

E. Formulations and Methods of Treatment

20 The morphogens, morphogen inducers, agonists of morphogen receptors, or small molecule morphogenic activators of the present invention may be provided to myogenic precursor cells by any suitable means, preferably directly (e.g., in vitro or locally after implantation, as by addition to culture medium, injection or topical administration to a tissue locus) or systemically (e.g., parenterally or orally). Preferably, the morphogen, morphogen inducer, agonist of a
25 morphogen receptor, or small molecule morphogenic activator comprises part of an aqueous, physiologically acceptable solution so that in addition to delivery of the desired agent to the target cells, the solution does not otherwise adversely affect the cells' or subject's electrolyte and/or volume balance. The aqueous medium for the agent thus may comprise normal physiologic saline (e.g., 9.85% NaCl, 0.15M, pH 7-7.4). Such an aqueous solution containing the agent can be
30 made, for example, by dissolving or dispersing the agent in 50% ethanol containing acetonitrile in

0.1% trifluoroacetic acid (TFA) or 0.1% HCl, or equivalent solvents. One volume of the resultant solution then is added, for example, to ten volumes of phosphate buffered saline (PBS), which further may include 0.1-0.2% human serum albumin (HSA). The resultant solution preferably is vortexed extensively.

5 For systemic administration, the morphogens, morphogen inducers, agonists of morphogen receptors, or small molecule morphogenic activators of the present invention may be administered by any route which is compatible with the particular morphogen, inducer, or agonist employed. Where the agent is to be provided parenterally, such as by intravenous, subcutaneous, intramuscular, intraorbital, ophthalmic, intraventricular, intracranial, intracapsular, intraspinal,
10 intracisternal, intraperitoneal, buccal, rectal, vaginal, intranasal or by aerosol administration, the agent preferably comprises part of an aqueous solution. In addition, administration may be by periodic injections of a bolus of the morphogen, inducer, agonist, or small molecule morphogenic activator, or may be made more continuous by intravenous or intraperitoneal administration from a reservoir which is external (e.g., an i.v. bag) or internal (e.g., a bioerodable implant, or a colony
15 of implanted, morphogen-producing cells).

If desired, a given morphogen or other agent may be made more soluble by association with a suitable molecule. For example, association of the mature morphogen dimer with the pro domain results in the pro form of the morphogen which typically is more soluble or dispersible in physiological solutions than the corresponding mature form. In fact, endogenous morphogens are
20 thought to be transported (e.g., secreted and circulated) in the mammalian body in this form. This soluble form of the protein can be obtained from culture medium of morphogen-secreting mammalian cells, e.g., cells transfected with nucleic acid encoding and competent to express the morphogen. Alternatively, a soluble species can be formulated by complexing the mature dimer (or an active fragment thereof) with a morphogen pro domain or a solubility-enhancing fragment
25 thereof (described more fully below). Another molecule capable of enhancing solubility and particularly useful for oral administrations, is casein. For example, addition of 0.2% casein increases solubility of the mature active form of OP1 by 80%. Other components found in milk and/or various serum proteins also may be useful.

Useful solutions for parenteral administration may be prepared by any of the methods well
30 known in the pharmaceutical art, described, for example, in Remington's Pharmaceutical Sciences

(Gennaro, A., ed.), Mack Pub., 1990. Formulations of the therapeutic agents of the invention may include, for example, polyalkylene glycols such as polyethylene glycol, oils of vegetable origin, hydrogenated naphthalenes, and the like. Formulations for direct administration, in particular, may include glycerol and other compositions of high viscosity to help maintain the agent at the desired locus. Biocompatible, preferably bioresorbable, polymers, including, for example, hyaluronic acid, collagen, tricalcium phosphate, polybutyrate, lactide, and glycolide polymers and lactide/glycolide copolymers, may be useful excipients to control the release of the agent *in vivo*. Other potentially useful parenteral delivery systems for these agents include ethylene-vinyl acetate copolymer particles, osmotic pumps, implantable infusion systems, and liposomes. Formulations for inhalation administration contain as excipients, for example, lactose, or may be aqueous solutions containing, for example, polyoxyethylene-9-lauryl ether, glycocholate and deoxycholate, or oily solutions for administration in the form of nasal drops, or as a gel to be applied intranasally. Formulations for parenteral administration may also include glycocholate for buccal administration, methoxysalicylate for rectal administration, or citric acid for vaginal administration. Suppositories for rectal administration also may be prepared by mixing the morphogen, inducer, agonist, or small molecule morphogenic activator with a non-irritating excipient such as cocoa butter or other compositions which are solid at room temperature and liquid at body temperatures.

Formulations for local or topical administration to a tissue or skin surface may be prepared by dispersing the morphogen, inducer, agonist or small molecule morphogenic activator with an acceptable carrier such as a lotion, cream, ointment or soap. Particularly useful are carriers capable of forming a film or layer over the skin or tissue to localize application and inhibit removal. For local or topical administration to internal tissue surfaces, the agent may be dispersed in a liquid tissue adhesive or other substance known to enhance adsorption to a tissue surface. For example, hydroxypropylcellulose or fibrinogen/thrombin solutions may be used to advantage. Alternatively, tissue-coating solutions, such as pectin-containing formulations may be used.

Alternatively, the agents described herein may be administered orally. Oral administration of proteins as therapeutics generally is not practiced as most proteins are readily degraded by digestive enzymes and acids in the mammalian digestive system before they can be absorbed into the bloodstream. However, the morphogens described herein typically are acid stable and

protease-resistant (see, for example, U.S. Pat. No. 4,968,590). In addition, at least one morphogen, OP1, has been identified in mammary gland extract, colostrum and 57-day milk. Moreover, the OP1 purified from mammary gland extract is morphogenically active and also is detected in the bloodstream. Maternal administration, via ingested milk, may be a natural delivery route of TGF β superfamily proteins. Letterio et al. (1994), Science 264:1936-1938, report that TGF β is present in murine milk, and that radiolabeled TGF β is absorbed by gastrointestinal mucosa of suckling juveniles. Labeled, ingested TGF β appears rapidly in intact form in the juveniles' body tissues, including lung, heart and liver. Finally, soluble form morphogen, e.g., mature morphogen associated with the pro domain, is morphogenically active. These findings, as well as those disclosed in the examples below, indicate that oral and parenteral administration are viable means for administering TGF β superfamily proteins, including the morphogens, to an individual. In addition, while the mature forms of certain morphogens described herein typically are sparingly soluble, the morphogen form found in milk (and mammary gland extract and colostrum) is readily soluble, probably by association of the mature, morphogenically active form with part or all of the pro domain of the intact sequence and/or by association with one or more milk components. Accordingly, the compounds provided herein also may be associated with molecules capable of enhancing their solubility in vitro or in vivo.

The compounds provided herein also may be associated with molecules capable of targeting the morphogen, inducer, agonist or small molecule morphogenic activator to the desired tissue. For example, an antibody, antibody fragment, or other binding protein that interacts specifically with a surface molecule on cells of the desired tissue, may be used. Useful targeting molecules may be designed, for example, using the single chain binding site technology disclosed, for example, in U.S. Pat. No. 5,091,513. Targeting molecules can be covalently or non-covalently associated with the morphogen, inducer, agonist, or small molecule morphogenic activator.

As will be appreciated by one of ordinary skill in the art, the formulated compositions contain therapeutically effective amounts of the morphogen, morphogen inducers, agonists of morphogen receptors, or small molecule morphogenic activators. That is, they contain amounts which provide appropriate concentrations of the agent to the mammalian myogenic precursor cells for a time sufficient to stimulate morphogenesis of new and functional myocardium, and/or to

prevent, inhibit or delay further significant loss of myocardium or decline of myocardial function. As will be appreciated by those skilled in the art, the concentration of the compounds described in a therapeutic composition of the present invention will vary depending upon a number of factors, including the biological efficacy of the selected agent, the chemical characteristics (e.g., hydrophobicity) of the compounds employed, the formulation of the compound excipients, the administration route, and the treatment envisioned, including whether the active ingredient will be administered directly to cells in vitro, directly into a tissue site, or systemically. The preferred dosage to be administered also is likely to depend on such variables such as the condition of the diseased or damaged tissues, and the overall health status of the particular subject.

As a general matter, for systemic administration, daily or weekly dosages of 0.00001-1000 mg of a morphogen are sufficient, with 0.0001-100 mg being preferable, and 0.001 to 10 mg being even more preferable. Alternatively, a daily or weekly dosage of 0.01-1000 µg/kg body weight, more preferably 0.1-100 µg/kg body weight, may be advantageously employed. Dosages are preferably administered continuously, but daily, multi-weekly, weekly or monthly dosages may also be employed. In addition, in order to facilitate frequent infusions, implantation of a semi-permanent stent (e.g., intravenous, intraperitoneal, intracisternal or intracapsular) may be advisable. It should be noted that no obvious morphogen induced pathological lesions arise when mature morphogen (e.g., OP1, 20 mg) is administered daily to normal growing rats for 21 consecutive days. Moreover, 10 mg systemic injections of morphogen (e.g., OP1) injected daily for 10 days into normal newborn mice does not produce any gross abnormalities.

The morphogens, inducers, agonists or small molecule morphogenic activators of the invention may, of course, be administered alone or in combination with other molecules known to be beneficial in the treatment of the conditions described herein. Thus, in other embodiments the present invention provides pharmaceutical compositions in which a morphogen, morphogen inducer, agonist of a morphogen receptor, or small molecule morphogenic activator is combined with other agents which promote or enhance the proliferation and differentiation of myogenic precursor cells into new and functional myocardium. Thus, the present invention provides pharmaceutical compositions comprising a morphogen, or morphogen inducer, or agonist of a morphogen receptor, or small molecule morphogenic activator, in combination with one or more of a "muscle extract," conditioned medium from differentiated myotubes grown in culture, bFGF,

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IGF, PDGF, LIF, ACTH, MSH, or G-CSF. In each such composition, the ratios or the morphogenic and mitogenic agents may be adjusted based upon their activities, as disclosed in the literature or as determined through simple experimentation, to provide a therapeutically effective dosage of each compound in a single unit dosage. The morphogenic and mitogenic agents in such a composition each preferably comprise at least about 1%, and more preferably more than 5% or 10%, of the dry weight of the composition. The compositions may, however, include other pharmaceutical carriers and active agents, as described above and, generally, in Remington's Pharmaceutical Sciences (Gennaro, A., ed.), Mack Pub., 1990, and, therefore, the morphogenic and mitogenic agents may each comprise a small fraction of the final weight of the pharmaceutical composition.

Practice of the invention, including additional preferred aspects and embodiments thereof, will be still more fully understood from the following examples, which are presented herein for illustration only and should not be construed as limiting the invention in any way.

Examples

Preparation of Soluble Morphogen Complexes

A currently preferred form of the morphogen useful herein, having improved solubility in aqueous solutions, is a dimeric morphogenic protein comprising at least the C-terminal seven cysteine domain characteristic of the morphogen family, complexed with a peptide comprising a pro region of a member of the morphogen family, or a solubility-enhancing fragment thereof, or an allelic, species or other sequence variant thereof. Preferably, the dimeric morphogenic protein is complexed with two pro region peptides. Also, the dimeric morphogenic protein preferably is noncovalently complexed with the pro region peptides. The pro region peptides preferably comprise at least the N-terminal eighteen amino acids that define the pro domain of a given naturally occurring morphogen, or an allelic or phylogenetic counterpart variant thereof. In other preferred embodiments, peptides defining substantially the full length pro domain are used.

Other soluble forms of morphogens include dimers of the uncleaved pro forms of these proteins, as well as "hemi-dimers" wherein one subunit of the dimer is an uncleaved pro form of

the protein, and the other subunit comprises the mature form of the protein, including truncated forms thereof, preferably noncovalently associated with a cleaved pro domain peptide.

As described above and in published application WO94/03600, the teachings of which are incorporated herein by reference, useful pro domains include the full length pro regions, as well as various truncated forms hereof, particularly truncated forms cleaved at proteolytic Arg-Xaa-Xaa-Arg cleavage sites within the pro domain polypeptide. For example, in OP1, possible pro sequences include sequences defined by residues 30-292 (full length form); 48-292; and 158-292. Soluble OP1 complex stability is best enhanced when the pro region comprises the full length form rather than a truncated form, such as the residues 48-292 truncated form, in that residues 30-47 show sequence homology to the N-terminal portions of other morphogens, and currently are believed to have particular utility in enhancing complex stability for all morphogens. Accordingly, currently preferred pro domains include peptides comprising at least the N-terminal fragment, e.g., amino acid residues 30-47 of a naturally occurring morphogen pro domain, or a biosynthetic variant thereof that retains the solubility and/or stability enhancing properties of the naturally-occurring peptide.

As will be appreciated by those having ordinary skill in the art, useful sequences encoding the pro region can be obtained from genetic sequences encoding known morphogens. Alternatively, chimeric pro regions can be constructed from the sequences of one or more known morphogens. Still another option is to create a synthetic sequence variant of one or more known pro region sequences.

In another preferred aspect, useful pro region peptides include polypeptide chains comprising an amino acid sequence encoded by a nucleic acid that hybridizes under stringent conditions with a DNA or RNA sequence encoding at least the N-terminal eighteen amino acids of the pro region sequence for OP1 or OP2, e.g., nucleotides 136-192 and 152-211 of SEQ ID NOs: 15 and 19, respectively.

A. Isolation from conditioned media or body fluid

Morphogens are expressed from mammalian cells as soluble complexes. Typically, however the complex is disassociated during purification, generally by exposure to denaturants often added to the purification solutions, such as detergents, alcohols, organic solvents, chaotropic agents and compounds added to reduce the pH of the solution. Provided below is a

currently preferred protocol for purifying the soluble proteins from conditioned media (or, optionally, a body fluid such as serum, cerebrospinal or peritoneal fluid), under non-denaturing conditions. The method is rapid, reproducible and yields isolated soluble morphogen complexes in substantially pure form.

5 Soluble morphogen complexes can be isolated from conditioned media using a simple, three step chromatographic protocol performed in the absence of denaturants. The protocol involves running the media (or body fluid) over an affinity column, followed by ion exchange and gel filtration chromatographies. The affinity column described below is a Zn-IMAC column. The present protocol has general applicability to the purification of a variety of morphogens, all of
10 which are anticipated to be isolatable using only minor modifications of the protocol described below. An alternative protocol also envisioned to have utility includes an immunoaffinity column, created using standard procedures and, for example, using antibody specific for a given morphogen pro domain (complexed, for example, to a protein A-conjugated Sepharose column). Protocols for developing immunoaffinity columns are well described in the art (see, for example,
15 Guide to Protein Purification, M. Deutscher, ed., Academic Press, San Diego, 1990, particularly sections VII and XI thereof).

In this study, OP1 was expressed in mammalian (CHO, Chinese hamster ovary) cells as described in the art (see, for example, international application US90/05903 (WO91/05802)). The CHO cell conditioned media containing 0.5% FBS was initially purified using Immobilized Metal-
20 Ion Affinity Chromatography (IMAC). The soluble OP1 complex from conditioned media binds very selectively to the Zn-IMAC resin and a high concentration of imidazole (50 mM imidazole, pH 8.0) is required for the effective elution of the bound complex. The Zn-IMAC step separates the soluble OP1 from the bulk of the contaminating serum proteins that elute in the flowthrough and 35 mM imidazole wash fractions. The Zn-IMAC purified soluble OP1 is next applied to an S-
25 Sepharose cation-exchange column equilibrated in 20 mM NaPO₄ (pH 7.0) with 50 mM NaCl. This S-Sepharose step serves to further purify and concentrate the soluble OP1 complex in preparation for the following gel filtration step. The protein was applied to a Sephacryl S-200HR column equilibrated in TBS. Using substantially the same protocol, soluble morphogens also can be isolated from one or more body fluids, including serum, cerebrospinal fluid or peritoneal fluid.

IMAC was performed using Chelating-Sepharose (Pharmacia) that had been charged with three column volumes of 0.2 M ZnSO_4 . The conditioned media was titrated to pH 7.0 and applied directly to the Zn-IMAC resin equilibrated in 20 mM HEPES (pH 7.0) with 500 mM NaCl. The Zn-IMAC resin was loaded with 80 mL of starting conditioned media per mL of resin. After loading, the column was washed with equilibration buffer and most of the contaminating proteins were eluted with 35 mM imidazole (pH 7.0) in equilibration buffer. The soluble OP1 complex then is eluted with 50 mM imidazole (pH 8.0) in 20 mM HEPES and 500 mM NaCl.

The 50 mM imidazole eluate containing the soluble OP1 complex was diluted with nine volumes of 20 mM NaPO_4 (pH 7.0) and applied to an S-Sepharose (Pharmacia) column equilibrated in 20 mM NaPO_4 (pH 7.0) with 50 mM NaCl. The S-Sepharose resin was loaded with an equivalent of 800 mL of starting conditioned media per mL of resin. After loading, the S-Sepharose column was washed with equilibration buffer and eluted with 100 mM NaCl followed by 300 mM and 500 mM NaCl in 20 mM NaPO_4 (pH 7.0). The 300 mM NaCl pool was further purified using gel filtration chromatography. Fifty mls of the 300 mM NaCl eluate was applied to a 5.0 X 90 cm Sephacryl S-200HR (Pharmacia) equilibrated in Tris buffered saline (TBS), 50 mM Tris, 150 mM NaCl (pH 7.4). The column was eluted at a flow rate of 5 mL/minute collecting 10 mL fractions. The apparent molecular mass of the soluble OP1 was determined by comparison to protein molecular weight standards (alcohol dehydrogenase (ADH, 150 kDa), bovine serum albumin (BSA, 68 kDa), carbonic anhydrase (CA, 30 kDa) and cytochrome C (cytC, 12.5 kDa). The purity of the S-200 column fractions was determined by separation on standard 15% polyacrylamide SDS gels stained with Coomassie blue. The identity of the mature OP1 and the pro-domain was determined by N-terminal sequence analysis after separation of the mature OP1 from the pro-domain using standard reverse phase C18 HPLC.

The soluble OP1 complex elutes with an apparent molecular weight of 110 kDa. This agrees well with the predicted composition of the soluble OP1 complex with one mature OP1 dimer (35-36 kDa) associated with two pro-domains (39 kDa each). Purity of the final complex can be verified by running the appropriate fraction in a reduced 15% polyacrylamide gel.

The complex components can be verified by running the complex-containing fraction from the S-200 or S-200HR columns over a reverse phase C18 HPLC column and eluting in an acetonitrile gradient (in 0.1% TFA), using standard procedures. The complex is dissociated by

this step, and the pro domain and mature species elute as separate species. These separate species then can be subjected to N-terminal sequencing using standard procedures (see, for example, Guide to Protein Purification, M. Deutscher, ed., Academic Press, San Diego, 1990, particularly pp. 602-613), and the identity of the isolated 36 kDa, 39 kDa proteins confirmed as mature morphogen and isolated, cleaved pro domain, respectively. N-terminal sequencing of the isolated pro domain from mammalian cell produced OP1 revealed two forms of the pro region, the intact form (beginning at residue 30 of SEQ ID NO: 16) and a truncated form, (beginning at residue 48 of SEQ ID NO: 16.) N-terminal sequencing of the polypeptide subunit of the isolated mature species reveals a range of N-termini for the mature sequence, beginning at residues 293, 300, 313, 315, 316, and 318, of SEQ ID NO: 16, all of which are active, as demonstrated by the standard bone morphogenesis assay set forth in published application WO92/15323 as incorporated herein by reference.

B. In Vitro Soluble Morphogen Complex Formation

As an alternative to purifying soluble complexes from culture media or a body fluid, soluble complexes can be formulated from purified pro domains and mature dimeric species. Successful complex formation apparently requires association of the components under denaturing conditions sufficient to relax the folded structure of these molecules, without affecting disulfide bonds. Preferably, the denaturing conditions mimic the environment of an intracellular vesicle sufficiently such that the cleaved pro domain has an opportunity to associate with the mature dimeric species under relaxed folding conditions. The concentration of denaturant in the solution then is decreased in a controlled, preferably step-wise manner, so as to allow proper refolding of the dimer and pro regions while maintaining the association of the pro domain with the dimer. Useful denaturants include 4-6M urea or guanidine hydrochloride (GuHCl), in buffered solutions of pH 4-10, preferably pH 6-8. The soluble complex then is formed by controlled dialysis or dilution into a solution having a final denaturant concentration of less than 0.1-2M urea or GuHCl, preferably 1-2 M urea or GuHCl, which then preferably can be diluted into a physiological buffer. Protein purification/renaturing procedures and considerations are well described in the art, and details for developing a suitable renaturing protocol readily can be determined by one having ordinary skill in the art. One useful text on the subject is Guide to

Protein Purification, M. Deutscher, ed., Academic Press, San Diego, 1990, particularly section V. Complex formation also may be aided by addition of one or more chaperone proteins.

C. Stability of Soluble Morphogen Complexes

The stability of the highly purified soluble morphogen complex in a physiological buffer, e.g., Tris-buffered saline (TBS) and phosphate-buffered saline (PBS), can be enhanced by any of a number of means. The currently preferred method is by means of a pro region that comprises at least the first 18 amino acids of the pro sequence (e.g., residues 30-47 of SEQ ID NO: 16 for OP-1), and preferably is the full length pro region. Residues 30-47 show sequence homology to the N-terminal portion of other morphogens and are believed to have particular utility in enhancing complex stability for all morphogens. Other useful means for enhancing the stability of soluble morphogen complexes include three classes of additives. These additives include basic amino acids (e.g., L-arginine, lysine and betaine); nonionic detergents (e.g., Tween 80 or Nonidet P-120); and carrier proteins (e.g., serum albumin and casein). Useful concentrations of these additives include 1-100 mM, preferably 10-70 mM, including 50 mM, basic amino acid, 0.01-1.0%, preferably 0.05-0.2%, including 0.1% (v/v) nonionic detergent, and 0.01-1.0%, preferably 0.05-0.2%, including 0.1% (w/v) carrier protein.

Equivalents

The invention may be embodied in other specific forms without departing from the spirit or essential characteristics thereof. The foregoing embodiments are therefore to be considered in all respects illustrative rather than limiting on the invention described herein. Scope of the invention is thus indicated by the appended claims rather than by the foregoing description, and all changes which come within the meaning and range of equivalency of the claims are intended to be embraced therein.

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SEQUENCE LISTING

(1) GENERAL INFORMATION:

- (i) APPLICANT: COHEN, CHARLES M.
- (ii) TITLE OF INVENTION: TREATMENT OF MAMMALIAN MYOCARDIUM WITH MORPHOGENICALLY-TREATED MYOGENIC PRECURSOR CELLS
- (iii) NUMBER OF SEQUENCES: 31
- (iv) CORRESPONDENCE ADDRESS:
 - (A) ADDRESSEE: TESTA, HURWITZ & THIBEAULT, LLP
 - (B) STREET: 125 HIGH STREET
 - (C) CITY: BOSTON
 - (D) STATE: MA
 - (E) COUNTRY: USA
 - (F) ZIP: 02110
- (v) COMPUTER READABLE FORM:
 - (A) MEDIUM TYPE: Floppy disk
 - (B) COMPUTER: IBM PC compatible
 - (C) OPERATING SYSTEM: PC-DOS/MS-DOS
 - (D) SOFTWARE: PatentIn Release #1.0, Version #1.25
- (vi) CURRENT APPLICATION DATA:
 - (A) APPLICATION NUMBER:
 - (B) FILING DATE:
 - (C) CLASSIFICATION:
- (viii) ATTORNEY/AGENT INFORMATION:
 - (A) NAME: TWOMEY, MICHAEL J
 - (B) REGISTRATION NUMBER: 38,349
 - (C) REFERENCE/DOCKET NUMBER: CRP-123
- (ix) TELECOMMUNICATION INFORMATION:
 - (A) TELEPHONE: 617/248-7000
 - (B) TELEFAX: 617/248-7100

(2) INFORMATION FOR SEQ ID NO:1:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 97 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: protein
- (ix) FEATURE:
 - (A) NAME/KEY: Protein
 - (B) LOCATION: 1..97
 - (D) OTHER INFORMATION: /label= Generic-Seq-7
/note= "wherein each Xaa is independently selected from a group of one or more specified amino acids as defined in the specification."
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

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Leu Xaa Xaa Xaa Phe Xaa Xaa Xaa Gly Trp Xaa Xaa Xaa Xaa Xaa Xaa
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Pro Xaa Xaa Xaa Xaa Ala Xaa Tyr Cys Xaa Gly Xaa Cys Xaa Xaa Pro
      20      25      30
Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Asn His Ala Xaa Xaa Xaa Xaa Xaa
      35      40      45
Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Cys Cys Xaa Pro
      50      55      60
Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Leu Xaa Xaa Xaa Xaa Xaa Xaa Xaa
      65      70      75      80
Val Xaa Leu Xaa Xaa Xaa Xaa Xaa Met Xaa Val Xaa Xaa Cys Xaa Cys
      85      90      95
Xaa

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(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 102 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(ix) FEATURE:

- (A) NAME/KEY: Protein
- (B) LOCATION: 1..102
- (D) OTHER INFORMATION: /label= Generic-Seq-8
/note= "wherin each Xaa is independently selected
from a group of one or more specified amino acids
as defined in the specification."

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

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1      5      10      15
Xaa Xaa Xaa Xaa Xaa Pro Xaa Xaa Xaa Xaa Ala Xaa Tyr Cys Xaa Gly
      20      25      30
Xaa Cys Xaa Xaa Pro Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Asn His Ala
      35      40      45
Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
      50      55      60
Xaa Cys Cys Xaa Pro Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Leu Xaa Xaa
      65      70      75      80
Xaa Xaa Xaa Xaa Xaa Val Xaa Leu Xaa Xaa Xaa Xaa Xaa Met Xaa Val
      85      90      95
Xaa Xaa Cys Xaa Cys Xaa
      100

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(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 102 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(ix) FEATURE:

- (A) NAME/KEY: Protein
- (B) LOCATION: 1..102
- (D) OTHER INFORMATION: /label= OPX
/note= "WHEREIN EACH XAA IS INDEPENDENTLY SELECTED
FROM A GROUP OF ONE OR MORE SPECIFIED AMINO ACIDS
AS DEFINED IN THE SPECIFICATION"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

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Cys Xaa Xaa His Glu Leu Tyr Val Xaa Phe Xaa Asp Leu Gly Trp Xaa
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Asp Trp Xaa Ile Ala Pro Xaa Gly Tyr Xaa Ala Tyr Tyr Cys Glu Gly
20          25          30
Glu Cys Xaa Phe Pro Leu Xaa Ser Xaa Met Asn Ala Thr Asn His Ala
35          40          45
Ile Xaa Gln Xaa Leu Val His Xaa Xaa Xaa Pro Xaa Xaa Val Pro Lys
50          55          60
Xaa Cys Cys Ala Pro Thr Xaa Leu Xaa Ala Xaa Ser Val Leu Tyr Xaa
65          70          75          80
Asp Xaa Ser Xaa Asn Val Xaa Leu Xaa Lys Xaa Arg Asn Met Val Val
85          90          95
Xaa Ala Cys Gly Cys His
100

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(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 139 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Homo sapiens
- (F) TISSUE TYPE: HIPPOCAMPUS

(ix) FEATURE:

- (A) NAME/KEY: Protein
- (B) LOCATION: 1..139

- 53 -

(D) OTHER INFORMATION: /label= hOP1-MATURE

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

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Ser Thr Gly Ser Lys Gln Arg Ser Gln Asn Arg Ser Lys Thr Pro Lys
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      20      25      30
Asp Gln Arg Gln Ala Cys Lys Lys His Glu Leu Tyr Val Ser Phe Arg
      35      40      45
Asp Leu Gly Trp Gln Asp Trp Ile Ile Ala Pro Glu Gly Tyr Ala Ala
      50      55      60
Tyr Tyr Cys Glu Gly Glu Cys Ala Phe Pro Leu Asn Ser Tyr Met Asn
      65      70      75      80
Ala Thr Asn His Ala Ile Val Gln Thr Leu Val His Phe Ile Asn Pro
      85      90      95
Glu Thr Val Pro Lys Pro Cys Cys Ala Pro Thr Gln Leu Asn Ala Ile
      100      105      110
Ser Val Leu Tyr Phe Asp Asp Ser Ser Asn Val Ile Leu Lys Lys Tyr
      115      120      125
Arg Asn Met Val Val Arg Ala Cys Gly Cys His
      130      135

```

(2) INFORMATION FOR SEQ ID NO:5:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 139 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: MURIDAE
- (F) TISSUE TYPE: EMBRYO

(ix) FEATURE:

- (A) NAME/KEY: Protein
- (B) LOCATION: 1..139
- (D) OTHER INFORMATION: /label= MOP1-MATURE

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

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Ser Thr Gly Gly Lys Gln Arg Ser Gln Asn Arg Ser Lys Thr Pro Lys
1      5      10      15
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      20      25      30
Asp Gln Arg Gln Ala Cys Lys Lys His Glu Leu Tyr Val Ser Phe Arg
      35      40      45

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Asp Leu Gly Trp Gln Asp Trp Ile Ile Ala Pro Glu Gly Tyr Ala Ala
 50 55 60
 Tyr Tyr Cys Glu Gly Glu Cys Ala Phe Pro Leu Asn Ser Tyr Met Asn
 65 70 75 80
 Ala Thr Asn His Ala Ile Val Gln Thr Leu Val His Phe Ile Asn Pro
 85 90 95
 Asp Thr Val Pro Lys Pro Cys Cys Ala Pro Thr Gln Leu Asn Ala Ile
 100 105 110
 Ser Val Leu Tyr Phe Asp Asp Ser Ser Asn Val Ile Leu Lys Lys Tyr
 115 120 125
 Arg Asn Met Val Val Arg Ala Cys Gly Cys His
 130 135

(2) INFORMATION FOR SEQ ID NO:6:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 139 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: protein
- (vi) ORIGINAL SOURCE:
 - (A) ORGANISM: HOMO SAPIENS
 - (F) TISSUE TYPE: HIPPOCAMPUS
- (ix) FEATURE:
 - (A) NAME/KEY: Protein
 - (B) LOCATION: 1..139
 - (D) OTHER INFORMATION: /label= HOP2-MATURE

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Ala Val Arg Pro Leu Arg Arg Arg Gln Pro Lys Lys Ser Asn Glu Leu
 1 5 10 15
 Pro Gln Ala Asn Arg Leu Pro Gly Ile Phe Asp Asp Val His Gly Ser
 20 25 30
 His Gly Arg Gln Val Cys Arg Arg His Glu Leu Tyr Val Ser Phe Gln
 35 40 45
 Asp Leu Gly Trp Leu Asp Trp Val Ile Ala Pro Gln Gly Tyr Ser Ala
 50 55 60
 Tyr Tyr Cys Glu Gly Glu Cys Ser Phe Pro Leu Asp Ser Cys Met Asn
 65 70 75 80
 Ala Thr Asn His Ala Ile Leu Gln Ser Leu Val His Leu Met Lys Pro
 85 90 95
 Asn Ala Val Pro Lys Ala Cys Cys Ala Pro Thr Lys Leu Ser Ala Thr
 100 105 110
 Ser Val Leu Tyr Tyr Asp Ser Ser Asn Asn Val Ile Leu Arg Lys His
 115 120 125

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Arg Asn Met Val Val Lys Ala Cys Gly Cys His
130 135

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 139 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: MURIDAE
- (F) TISSUE TYPE: EMBRYO

(ix) FEATURE:

- (A) NAME/KEY: Protein
- (B) LOCATION: 1..139
- (D) OTHER INFORMATION: /label= MOP2-MATURE

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

Ala Ala Arg Pro Leu Lys Arg Arg Gln Pro Lys Lys Thr Asn Glu Leu
1 5 10 15
Pro His Pro Asn Lys Leu Pro Gly Ile Phe Asp Asp Gly His Gly Ser
20 25 30
Arg Gly Arg Glu Val Cys Arg Arg His Glu Leu Tyr Val Ser Phe Arg
35 40 45
Asp Leu Gly Trp Leu Asp Trp Val Ile Ala Pro Gln Gly Tyr Ser Ala
50 55 60
Tyr Tyr Cys Glu Gly Glu Cys Ala Phe Pro Leu Asp Ser Cys Met Asn
65 70 75 80
Ala Thr Asn His Ala Ile Leu Gln Ser Leu Val His Leu Met Lys Pro
85 90 95
Asp Val Val Pro Lys Ala Cys Cys Ala Pro Thr Lys Leu Ser Ala Thr
100 105 110
Ser Val Leu Tyr Tyr Asp Ser Ser Asn Asn Val Ile Leu Arg Lys His
115 120 125
Arg Asn Met Val Val Lys Ala Cys Gly Cys His
130 135

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 101 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

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(vi) ORIGINAL SOURCE:

(A) ORGANISM: bovinæ

(ix) FEATURE:

(A) NAME/KEY: Protein

(B) LOCATION: 1..101

(D) OTHER INFORMATION: /label= CBMP-2A-FX

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

Cys Lys Arg His Pro Leu Tyr Val Asp Phe Ser Asp Val Gly Trp Asn
 1 5 10 15
 Asp Trp Ile Val Ala Pro Pro Gly Tyr His Ala Phe Tyr Cys His Gly
 20 25 30
 Glu Cys Pro Phe Pro Leu Ala Asp His Leu Asn Ser Thr Asn His Ala
 35 40 45
 Ile Val Gln Thr Leu Val Asn Ser Val Asn Ser Lys Ile Pro Lys Ala
 50 55 60
 Cys Cys Val Pro Thr Glu Leu Ser Ala Ile Ser Met Leu Tyr Leu Asp
 65 70 75 80
 Glu Asn Glu Lys Val Val Leu Lys Asn Tyr Gln Asp Met Val Val Glu
 85 90 95
 Gly Cys Gly Cys Arg
 100

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 101 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(vi) ORIGINAL SOURCE:

(A) ORGANISM: HOMO SAPIENS

(F) TISSUE TYPE: hippocampus

(ix) FEATURE:

(A) NAME/KEY: Protein

(B) LOCATION: 1..101

(D) OTHER INFORMATION: /label= CBMP-2B-FX

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

Cys Arg Arg His Ser Leu Tyr Val Asp Phe Ser Asp Val Gly Trp Asn
 1 5 10 15
 Asp Trp Ile Val Ala Pro Pro Gly Tyr Gln Ala Phe Tyr Cys His Gly
 20 25 30
 Asp Cys Pro Phe Pro Leu Ala Asp His Leu Asn Ser Thr Asn His Ala
 35 40 45

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Ile Val Gln Thr Leu Val Asn Ser Val Asn Ser Ser Ile Pro Lys Ala
 50 55 60
 Cys Cys Val Pro Thr Glu Leu Ser Ala Ile Ser Met Leu Tyr Leu Asp
 65 70 75 80
 Glu Tyr Asp Lys Val Val Leu Lys Asn Tyr Gln Glu Met Val Val Glu
 85 90 95
 Gly Cys Gly Cys Arg
 100

(2) INFORMATION FOR SEQ ID NO:10:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 102 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: protein
- (vi) ORIGINAL SOURCE:
 - (A) ORGANISM: DROSOPHILA MELANOGASTER
- (ix) FEATURE:
 - (A) NAME/KEY: Protein
 - (B) LOCATION: 1..101
 - (D) OTHER INFORMATION: /label= DPP-FX

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

Cys Arg Arg His Ser Leu Tyr Val Asp Phe Ser Asp Val Gly Trp Asp
 1 5 10 15
 Asp Trp Ile Val Ala Pro Leu Gly Tyr Asp Ala Tyr Tyr Cys His Gly
 20 25 30
 Lys Cys Pro Phe Pro Leu Ala Asp His Phe Asn Ser Thr Asn His Ala
 35 40 45
 Val Val Gln Thr Leu Val Asn Asn Asn Asn Pro Gly Lys Val Pro Lys
 50 55 60
 Ala Cys Cys Val Pro Thr Gln Leu Asp Ser Val Ala Met Leu Tyr Leu
 65 70 75 80
 Asn Asp Gln Ser Thr Val Val Leu Lys Asn Tyr Gln Glu Met Thr Val
 85 90 95
 Val Gly Cys Gly Cys Arg
 100

(2) INFORMATION FOR SEQ ID NO:11:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 102 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: protein

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(vi) ORIGINAL SOURCE:

(A) ORGANISM: XENOPUS

(ix) FEATURE:

(A) NAME/KEY: Protein

(B) LOCATION: 1..102

(D) OTHER INFORMATION: /label= VGL-FX

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

Cys Lys Lys Arg His Leu Tyr Val Glu Phe Lys Asp Val Gly Trp Gln
 1 5 10 15
 Asn Trp Val Ile Ala Pro Gln Gly Tyr Met Ala Asn Tyr Cys Tyr Gly
 20 25 30
 Glu Cys Pro Tyr Pro Leu Thr Glu Ile Leu Asn Gly Ser Asn His Ala
 35 40 45
 Ile Leu Gln Thr Leu Val His Ser Ile Glu Pro Glu Asp Ile Pro Leu
 50 55 60
 Pro Cys Cys Val Pro Thr Lys Met Ser Pro Ile Ser Met Leu Phe Tyr
 65 70 75 80
 Asp Asn Asn Asp Asn Val Val Leu Arg His Tyr Glu Asn Met Ala Val
 85 90 95
 Asp Glu Cys Gly Cys Arg
 100

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 102 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(vi) ORIGINAL SOURCE:

(A) ORGANISM: MURIDAE

(ix) FEATURE:

(A) NAME/KEY: Protein

(B) LOCATION: 1..102

(D) OTHER INFORMATION: /label= VGR-1-FX

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

Cys Lys Lys His Glu Leu Tyr Val Ser Phe Gln Asp Val Gly Trp Gln
 1 5 10 15
 Asp Trp Ile Ile Ala Pro Lys Gly Tyr Ala Ala Asn Tyr Cys Asp Gly
 20 25 30
 Glu Cys Ser Phe Pro Leu Asn Ala His Met Asn Ala Thr Asn His Ala
 35 40 45

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Ile Val Gln Thr Leu Val His Val Met Asn Pro Glu Tyr Val Pro Lys
 50 55 60
 Pro Cys Cys Ala Pro Thr Lys Val Asn Ala Ile Ser Val Leu Tyr Phe
 65 70 75 80
 Asp Asp Asn Ser Asn Val Ile Leu Lys Lys Tyr Arg Asn Met Val Val
 85 90 95
 Arg Ala Cys Gly Cys His
 100

(2) INFORMATION FOR SEQ ID NO:13:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 106 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: protein
- (iii) HYPOTHETICAL: NO
- (iv) ANTI-SENSE: NO
- (vi) ORIGINAL SOURCE:
 - (A) ORGANISM: Homo sapiens
 - (F) TISSUE TYPE: brain
- (ix) FEATURE:
 - (A) NAME/KEY: Protein
 - (B) LOCATION: 1..106
 - (D) OTHER INFORMATION: /note= "GDF-1 (fx)"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

Cys Arg Ala Arg Arg Leu Tyr Val Ser Phe Arg Glu Val Gly Trp His
 1 5 10 15
 Arg Trp Val Ile Ala Pro Arg Gly Phe Leu Ala Asn Tyr Cys Gln Gly
 20 25 30
 Gln Cys Ala Leu Pro Val Ala Leu Ser Gly Ser Gly Gly Pro Pro Ala
 35 40 45
 Leu Asn His Ala Val Leu Arg Ala Leu Met His Ala Ala Ala Pro Gly
 50 55 60
 Ala Ala Asp Leu Pro Cys Cys Val Pro Ala Arg Leu Ser Pro Ile Ser
 65 70 75 80
 Val Leu Phe Phe Asp Asn Ser Asp Asn Val Val Leu Arg Gln Tyr Glu
 85 90 95
 Asp Met Val Val Asp Glu Cys Gly Cys Arg
 100 105

(2) INFORMATION FOR SEQ ID NO:14:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 5 amino acids

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- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

Cys Xaa Xaa Xaa Xaa
1 5

(2) INFORMATION FOR SEQ ID NO:15:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 1822 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: cDNA
- (iii) HYPOTHETICAL: NO
- (iv) ANTI-SENSE: NO
- (vi) ORIGINAL SOURCE:
 - (A) ORGANISM: HOMO SAPIENS
 - (F) TISSUE TYPE: HIPPOCAMPUS
- (ix) FEATURE:
 - (A) NAME/KEY: CDS
 - (B) LOCATION: 49..1341
 - (C) IDENTIFICATION METHOD: experimental
 - (D) OTHER INFORMATION: /function= "OSTEOGENIC PROTEIN"
/product= "OP1"
/evidence= EXPERIMENTAL
/standard_name= "OP1"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

GGTGCGGGCC CGGAGCCCGG AGCCCGGGTA GCGCGTAGAG CCGGCGCG ATG CAC GTG	57
Met His Val	
1	
CGC TCA CTG CGA GCT GCG GCG CCG CAC AGC TTC GTG GCG CTC TGG GCA	105
Arg Ser Leu Arg Ala Ala Ala Pro His Ser Phe Val Ala Leu Trp Ala	
5 10 15	
CCC CTG TTC CTG CTG CGC TCC GCC CTG GCC GAC TTC AGC CTG GAC AAC	153
Pro Leu Phe Leu Leu Arg Ser Ala Leu Ala Asp Phe Ser Leu Asp Asn	
20 25 30 35	
GAG GTG CAC TCG AGC TTC ATC CAC CGG CGC CTC CGC AGC CAG GAG CGG	201
Glu Val His Ser Ser Phe Ile His Arg Arg Leu Arg Ser Gln Glu Arg	
40 45 50	
CGG GAG ATG CAG CGC GAG ATC CTC TCC ATT TTG GGC TTG CCC CAC CGC	249
Arg Glu Met Gln Arg Glu Ile Leu Ser Ile Leu Gly Leu Pro His Arg	
55 60 65	

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CCG	CGC	CCG	CAC	CTC	CAG	GGC	AAG	CAC	AAC	TCG	GCA	CCC	ATG	TTC	ATG	297
Pro	Arg	Pro	His	Leu	Gln	Gly	Lys	His	Asn	Ser	Ala	Pro	Met	Phe	Met	
		70					75					80				
CTG	GAC	CTG	TAC	AAC	GCC	ATG	GCG	GTG	GAG	GAG	GGC	GGC	GGG	CCC	GGC	345
Leu	Asp	Leu	Tyr	Asn	Ala	Met	Ala	Val	Glu	Glu	Gly	Gly	Gly	Pro	Gly	
	85					90					95					
GGC	CAG	GGC	TTC	TCC	TAC	CCC	TAC	AAG	GCC	GTC	TTC	AGT	ACC	CAG	GGC	393
Gly	Gln	Gly	Phe	Ser	Tyr	Pro	Tyr	Lys	Ala	Val	Phe	Ser	Thr	Gln	Gly	
100					105					110					115	
CCC	CCT	CTG	GCC	AGC	CTG	CAA	GAT	AGC	CAT	TTC	CTC	ACC	GAC	GCC	GAC	441
Pro	Pro	Leu	Ala	Ser	Leu	Gln	Asp	Ser	His	Phe	Leu	Thr	Asp	Ala	Asp	
				120					125					130		
ATG	GTC	ATG	AGC	TTC	GTC	AAC	CTC	GTG	GAA	CAT	GAC	AAG	GAA	TTC	TTC	489
Met	Val	Met	Ser	Phe	Val	Asn	Leu	Val	Glu	His	Asp	Lys	Glu	Phe	Phe	
			135				140					145				
CAC	CCA	CGC	TAC	CAC	CAT	CGA	GAG	TTC	CGG	TTT	GAT	CTT	TCC	AAG	ATC	537
His	Pro	Arg	Tyr	His	His	Arg	Glu	Phe	Arg	Phe	Asp	Leu	Ser	Lys	Ile	
		150					155					160				
CCA	GAA	GGG	GAA	GCT	GTC	ACG	GCA	GCC	GAA	TTC	CGG	ATC	TAC	AAG	GAC	585
Pro	Glu	Gly	Glu	Ala	Val	Thr	Ala	Ala	Glu	Phe	Arg	Ile	Tyr	Lys	Asp	
	165					170					175					
TAC	ATC	CGG	GAA	CGC	TTC	GAC	AAT	GAG	ACG	TTC	CGG	ATC	AGC	GTT	TAT	633
Tyr	Ile	Arg	Glu	Arg	Phe	Asp	Asn	Glu	Thr	Phe	Arg	Ile	Ser	Val	Tyr	
180					185					190					195	
CAG	GTG	CTC	CAG	GAG	CAC	TTG	GGC	AGG	GAA	TCG	GAT	CTC	TTC	CTG	CTC	681
Gln	Val	Leu	Gln	Glu	His	Leu	Gly	Arg	Glu	Ser	Asp	Leu	Phe	Leu	Leu	
				200					205					210		
GAC	AGC	CGT	ACC	CTC	TGG	GCC	TCG	GAG	GAG	GGC	TGG	CTG	GTG	TTT	GAC	729
Asp	Ser	Arg	Thr	Leu	Trp	Ala	Ser	Glu	Glu	Gly	Trp	Leu	Val	Phe	Asp	
			215					220				225				
ATC	ACA	GCC	ACC	AGC	AAC	CAC	TGG	GTG	GTC	AAT	CCG	CGG	CAC	AAC	CTG	777
Ile	Thr	Ala	Thr	Ser	Asn	His	Trp	Val	Val	Asn	Pro	Arg	His	Asn	Leu	
		230					235					240				
GGC	CTG	CAG	CTC	TCG	GTG	GAG	ACG	CTG	GAT	GGG	CAG	AGC	ATC	AAC	CCC	825
Gly	Leu	Gln	Leu	Ser	Val	Glu	Thr	Leu	Asp	Gly	Gln	Ser	Ile	Asn	Pro	
	245					250					255					
AAG	TTG	GCG	GGC	CTG	ATT	GGG	CGG	CAC	GGG	CCC	CAG	AAC	AAG	CAG	CCC	873
Lys	Leu	Ala	Gly	Leu	Ile	Gly	Arg	His	Gly	Pro	Gln	Asn	Lys	Gln	Pro	
260					265					270					275	
TTC	ATG	GTG	GCT	TTC	TTC	AAG	GCC	ACG	GAG	GTC	CAC	TTC	CGC	AGC	ATC	921
Phe	Met	Val	Ala	Phe	Phe	Lys	Ala	Thr	Glu	Val	His	Phe	Arg	Ser	Ile	
				280					285					290		
CGG	TCC	ACG	GGG	AGC	AAA	CAG	CGC	AGC	CAG	AAC	CGC	TCC	AAG	ACG	CCC	969
Arg	Ser	Thr	Gly	Ser	Lys	Gln	Arg	Ser	Gln	Asn	Arg	Ser	Lys	Thr	Pro	
			295					300				305				
AAG	AAC	CAG	GAA	GCC	CTG	CGG	ATG	GCC	AAC	GTG	GCA	GAG	AAC	AGC	AGC	1017
Lys	Asn	Gln	Glu	Ala	Leu	Arg	Met	Ala	Asn	Val	Ala	Glu	Asn	Ser	Ser	
		310					315					320				

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AGC GAC CAG AGG CAG GCC TGT AAG AAG CAC GAG CTG TAT GTC AGC TTC Ser Asp Gln Arg Gln Ala Cys Lys Lys His Glu Leu Tyr Val Ser Phe 325 330 335	1065
CGA GAC CTG GGC TGG CAG GAC TGG ATC ATC GCG CCT GAA GGC TAC GCC Arg Asp Leu Gly Trp Gln Asp Trp Ile Ile Ala Pro Glu Gly Tyr Ala 340 345 350 355	1113
GCC TAC TAC TGT GAG GGG GAG TGT GCC TTC CCT CTG AAC TCC TAC ATG Ala Tyr Tyr Cys Glu Gly Glu Cys Ala Phe Pro Leu Asn Ser Tyr Met 360 365 370	1161
AAC GCC ACC AAC CAC GCC ATC GTG CAG ACG CTG GTC CAC TTC ATC AAC Asn Ala Thr Asn His Ala Ile Val Gln Thr Leu Val His Phe Ile Asn 375 380 385	1209
CCG GAA ACG GTG CCC AAG CCC TGC TGT GCG CCC ACG CAG CTC AAT GCC Pro Glu Thr Val Pro Lys Pro Cys Cys Ala Pro Thr Gln Leu Asn Ala 390 395 400	1257
ATC TCC GTC CTC TAC TTC GAT GAC AGC TCC AAC GTC ATC CTG AAG AAA Ile Ser Val Leu Tyr Phe Asp Asp Ser Ser Asn Val Ile Leu Lys Lys 405 410 415	1305
TAC AGA AAC ATG GTG GTC CGG GCC TGT GGC TGC CAC TAGCTCCTCC Tyr Arg Asn Met Val Val Arg Ala Cys Gly Cys His 420 425 430	1351
GAGAATTCAG ACCCTTTGGG GCCAAGTTTT TCTGGATCCT CCATTGCTCG CCTTGGCCAG	1411
GAACCAGCAG ACCAACTGCC TTTTGTGAGA CCTTCCCCTC CCTATCCCCA ACTTTAAAGG	1471
TGTGAGAGTA TTAGGAAACA TGAGCAGCAT ATGGCTTTTG ATCAGTTTTT CAGTGGCAGC	1531
ATCCAATGAA CAAGATCCTA CAAGCTGTGC AGGCAAAACC TAGCAGGAAA AAAAAACAAC	1591
GCATAAGAA AAATGGCCGG GCCAGGTCAT TGGCTGGGAA GTCTCAGCCA TGCACGGACT	1651
CGTTTCCAGA GGTAATTATG AGCGCCTACC AGCCAGGCCA CCCAGCCGTG GGAGGAAGGG	1711
GGCGTGGCAA GGGGTGGGCA CATTGGTGTC TGTGCGAAAG GAAAATTGAC CCGGAAGTTC	1771
CTGTAATAAA TGTCACAATA AAACGAATGA ATGAAAAAAA AAAAAAAAAA A	1822

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 431 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

Met	His	Val	Arg	Ser	Leu	Arg	Ala	Ala	Ala	Pro	His	Ser	Phe	Val	Ala
1				5					10					15	
Leu	Trp	Ala	Pro	Leu	Phe	Leu	Leu	Arg	Ser	Ala	Leu	Ala	Asp	Phe	Ser
		20						25					30		

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Ser Tyr Met Asn Ala Thr Asn His Ala Ile Val Gln Thr Leu Val His
 370 375 380

Phe Ile Asn Pro Glu Thr Val Pro Lys Pro Cys Cys Ala Pro Thr Gln
 385 390 395 400

Leu Asn Ala Ile Ser Val Leu Tyr Phe Asp Asp Ser Ser Asn Val Ile
 405 410 415

Leu Lys Lys Tyr Arg Asn Met Val Val Arg Ala Cys Gly Cys His
 420 425 430

(2) INFORMATION FOR SEQ ID NO:17:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 1873 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: cDNA
- (iii) HYPOTHETICAL: NO
- (iv) ANTI-SENSE: NO
- (vi) ORIGINAL SOURCE:
 - (A) ORGANISM: MURIDAE
 - (F) TISSUE TYPE: EMBRYO
- (ix) FEATURE:
 - (A) NAME/KEY: CDS
 - (B) LOCATION: 104..1393
 - (D) OTHER INFORMATION: /function= "OSTEOGENIC PROTEIN"
 /product= "MOP1"
 /note= "MOP1 (CDNA)"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

CTGCAGCAAG TGACCTCGGG TCGTGGACCG CTGCCCTGCC CCCTCCGCTG CCACCTGGGG 60

CGGCGCGGGC CCGGTGCCCC GGATCGCGCG TAGAGCCGGC GCG ATG CAC GTG CGC 115
 Met His Val Arg
 1

TCG CTG CGC GCT GCG GCG CCA CAC AGC TTC GTG GCG CTC TGG GCG CCT 163
 Ser Leu Arg Ala Ala Ala Pro His Ser Phe Val Ala Leu Trp Ala Pro
 5 10 15 20

CTG TTC TTG CTG CGC TCC GCC CTG GCC GAT TTC AGC CTG GAC AAC GAG 211
 Leu Phe Leu Leu Arg Ser Ala Leu Ala Asp Phe Ser Leu Asp Asn Glu
 25 30 35

GTG CAC TCC AGC TTC ATC CAC CGG CGC CTC CGC AGC CAG GAG CGG CGG 259
 Val His Ser Ser Phe Ile His Arg Arg Leu Arg Ser Gln Glu Arg Arg
 40 45 50

GAG ATG CAG CGG GAG ATC CTG TCC ATC TTA GGG TTG CCC CAT CGC CCG 307
 Glu Met Gln Arg Glu Ile Leu Ser Ile Leu Gly Leu Pro His Arg Pro
 55 60 65

CGC CCG CAC CTC CAG GGA AAG CAT AAT TCG GCG CCC ATG TTC ATG TTG 355

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Arg	Pro	His	Leu	Gln	Gly	Lys	His	Asn	Ser	Ala	Pro	Met	Phe	Met	Leu	
70						75					80					
GAC	CTG	TAC	AAC	GCC	ATG	GCG	GTG	GAG	GAG	AGC	GGG	CCG	GAC	GGA	CAG	403
Asp	Leu	Tyr	Asn	Ala	Met	Ala	Val	Glu	Glu	Ser	Gly	Pro	Asp	Gly	Gln	
85					90					95					100	
GGC	TTC	TCC	TAC	CCC	TAC	AAG	GCC	GTC	TTC	AGT	ACC	CAG	GGC	CCC	CCT	451
Gly	Phe	Ser	Tyr	Pro	Tyr	Lys	Ala	Val	Phe	Ser	Thr	Gln	Gly	Pro	Pro	
				105					110					115		
TTA	GCC	AGC	CTG	CAG	GAC	AGC	CAT	TTC	CTC	ACT	GAC	GCC	GAC	ATG	GTC	499
Leu	Ala	Ser	Leu	Gln	Asp	Ser	His	Phe	Leu	Thr	Asp	Ala	Asp	Met	Val	
			120					125					130			
ATG	AGC	TTC	GTC	AAC	CTA	GTG	GAA	CAT	GAC	AAA	GAA	TTC	TTC	CAC	CCT	547
Met	Ser	Phe	Val	Asn	Leu	Val	Glu	His	Asp	Lys	Glu	Phe	Phe	His	Pro	
		135					140					145				
CGA	TAC	CAC	CAT	CGG	GAG	TTC	CGG	TTT	GAT	CTT	TCC	AAG	ATC	CCC	GAG	595
Arg	Tyr	His	His	Arg	Glu	Phe	Arg	Phe	Asp	Leu	Ser	Lys	Ile	Pro	Glu	
	150					155					160					
GGC	GAA	CGG	GTG	ACC	GCA	GCC	GAA	TTC	AGG	ATC	TAT	AAG	GAC	TAC	ATC	643
Gly	Glu	Arg	Val	Thr	Ala	Ala	Glu	Phe	Arg	Ile	Tyr	Lys	Asp	Tyr	Ile	
165					170				175						180	
CGG	GAG	CGA	TTT	GAC	AAC	GAG	ACC	TTC	CAG	ATC	ACA	GTC	TAT	CAG	GTG	691
Arg	Glu	Arg	Phe	Asp	Asn	Glu	Thr	Phe	Gln	Ile	Thr	Val	Tyr	Gln	Val	
				185					190					195		
CTC	CAG	GAG	CAC	TCA	GGC	AGG	GAG	TCG	GAC	CTC	TTC	TTG	CTG	GAC	AGC	739
Leu	Gln	Glu	His	Ser	Gly	Arg	Glu	Ser	Asp	Leu	Phe	Leu	Leu	Asp	Ser	
				200				205					210			
CGC	ACC	ATC	TGG	GCT	TCT	GAG	GAG	GGC	TGG	TTG	GTG	TTT	GAT	ATC	ACA	787
Arg	Thr	Ile	Trp	Ala	Ser	Glu	Glu	Gly	Trp	Leu	Val	Phe	Asp	Ile	Thr	
		215					220					225				
GCC	ACC	AGC	AAC	CAC	TGG	GTG	GTC	AAC	CCT	CGG	CAC	AAC	CTG	GGC	TTA	835
Ala	Thr	Ser	Asn	His	Trp	Val	Val	Asn	Pro	Arg	His	Asn	Leu	Gly	Leu	
	230					235					240					
CAG	CTC	TCT	GTG	GAG	ACC	CTG	GAT	GGG	CAG	AGC	ATC	AAC	CCC	AAG	TTG	883
Gln	Leu	Ser	Val	Glu	Thr	Leu	Asp	Gly	Gln	Ser	Ile	Asn	Pro	Lys	Leu	
245					250					255					260	
GCA	GGC	CTG	ATT	GGA	CGG	CAT	GGA	CCC	CAG	AAC	AAG	CAA	CCC	TTC	ATG	931
Ala	Gly	Leu	Ile	Gly	Arg	His	Gly	Pro	Gln	Asn	Lys	Gln	Pro	Phe	Met	
				265					270					275		
GTG	GCC	TTC	TTC	AAG	GCC	ACG	GAA	GTC	CAT	CTC	CGT	AGT	ATC	CGG	TCC	979
Val	Ala	Phe	Phe	Lys	Ala	Thr	Glu	Val	His	Leu	Arg	Ser	Ile	Arg	Ser	
			280					285						290		
ACG	GGG	GGC	AAG	CAG	CGC	AGC	CAG	AAT	CGC	TCC	AAG	ACG	CCA	AAG	AAC	1027
Thr	Gly	Gly	Lys	Gln	Arg	Ser	Gln	Asn	Arg	Ser	Lys	Thr	Pro	Lys	Asn	
		295					300					305				
CAA	GAG	GCC	CTG	AGG	ATG	GCC	AGT	GTG	GCA	GAA	AAC	AGC	AGC	AGT	GAC	1075
Gln	Glu	Ala	Leu	Arg	Met	Ala	Ser	Val	Ala	Glu	Asn	Ser	Ser	Ser	Asp	
	310					315					320					

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CAG AGG CAG GCC TGC AAG AAA CAT GAG CTG TAC GTC AGC TTC CGA GAC Gln Arg Gln Ala Cys Lys Lys His Glu Leu Tyr Val Ser Phe Arg Asp 325 330 335 340	1123
CTT GGC TGG CAG GAC TGG ATC ATT GCA CCT GAA GGC TAT GCT GCC TAC Leu Gly Trp Gln Asp Trp Ile Ile Ala Pro Glu Gly Tyr Ala Ala Tyr 345 350 355	1171
TAC TGT GAG GGA GAG TGC GCC TTC CCT CTG AAC TCC TAC ATG AAC GCC Tyr Cys Glu Gly Glu Cys Ala Phe Pro Leu Asn Ser Tyr Met Asn Ala 360 365 370	1219
ACC AAC CAC GCC ATC GTC CAG ACA CTG GTT CAC TTC ATC AAC CCA GAC Thr Asn His Ala Ile Val Gln Thr Leu Val His Phe Ile Asn Pro Asp 375 380 385	1267
ACA GTA CCC AAG CCC TGC TGT GCG CCC ACC CAG CTC AAC GCC ATC TCT Thr Val Pro Lys Pro Cys Cys Ala Pro Thr Gln Leu Asn Ala Ile Ser 390 395 400	1315
GTC CTC TAC TTC GAC GAC AGC TCT AAT GTC ATC CTG AAG AAG TAC AGA Val Leu Tyr Phe Asp Asp Ser Ser Asn Val Ile Leu Lys Lys Tyr Arg 405 410 415 420	1363
AAC ATG GTG GTC CGG GCC TGT GGC TGC CAC TAGCTCTTCC TGAGACCCTG Asn Met Val Val Arg Ala Cys Gly Cys His 425 430	1413
ACCTTTGCGG GGCCACACCT TTCCAAATCT TCGATGTCTC ACCATCTAAG TCTCTCACTG	1473
CCCACCTTGG CGAGGAGAAC AGACCAACCT CTCCTGAGCC TTCCCTCACC TCCCAACCGG	1533
AAGCATGTAA GGGTTCAGAA AACCTGAGCG TGCAGCAGCT GATGAGCGCC CTTTCCTTCT	1593
GGCAGGTGAC GGACAAGATC CTACCAGCTA CCACAGCAAA CGCCTAAGAG CAGGAAAAAT	1653
GTCGCCCAGG AAAGTGTCCA GTGTCCACAT GGCCCCTGGC GCTCTGAGTC TTTGAGGAGT	1713
AATCGCAAGC CTCGTTTCAGC TGCAGCAGAA GGAAGGGCTT AGCCAGGGTG GGCGCTGGCG	1773
TCTGTGTTGA AGGGAAACCA AGCAGAAGCC ACTGTAATGA TATGTCACAA TAAAACCCAT	1833
GAATGAAAAA AAAAAAAAAA AAAAAAAAAA AAAAGAATTC	1873

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 430 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

Met His Val Arg Ser Leu Arg Ala Ala Ala Pro His Ser Phe Val Ala
1 5 10 15

Leu Trp Ala Pro Leu Phe Leu Leu Arg Ser Ala Leu Ala Asp Phe Ser
20 25 30

Leu Asp Asn Glu Val His Ser Ser Phe Ile His Arg Arg Leu Arg Ser

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35 40 45
 Gln Glu Arg Arg Glu Met Gln Arg Glu Ile Leu Ser Ile Leu Gly Leu
 50 55 60
 Pro His Arg Pro Arg Pro His Leu Gln Gly Lys His Asn Ser Ala Pro
 65 70 75 80
 Met Phe Met Leu Asp Leu Tyr Asn Ala Met Ala Val Glu Glu Ser Gly
 85 90 95
 Pro Asp Gly Gln Gly Phe Ser Tyr Pro Tyr Lys Ala Val Phe Ser Thr
 100 105 110
 Gln Gly Pro Pro Leu Ala Ser Leu Gln Asp Ser His Phe Leu Thr Asp
 115 120 125
 Ala Asp Met Val Met Ser Phe Val Asn Leu Val Glu His Asp Lys Glu
 130 135 140
 Phe Phe His Pro Arg Tyr His His Arg Glu Phe Arg Phe Asp Leu Ser
 145 150 155 160
 Lys Ile Pro Glu Gly Glu Arg Val Thr Ala Ala Glu Phe Arg Ile Tyr
 165 170 175
 Lys Asp Tyr Ile Arg Glu Arg Phe Asp Asn Glu Thr Phe Gln Ile Thr
 180 185 190
 Val Tyr Gln Val Leu Gln Glu His Ser Gly Arg Glu Ser Asp Leu Phe
 195 200 205
 Leu Leu Asp Ser Arg Thr Ile Trp Ala Ser Glu Glu Gly Trp Leu Val
 210 215 220
 Phe Asp Ile Thr Ala Thr Ser Asn His Trp Val Val Asn Pro Arg His
 225 230 235 240
 Asn Leu Gly Leu Gln Leu Ser Val Glu Thr Leu Asp Gly Gln Ser Ile
 245 250 255
 Asn Pro Lys Leu Ala Gly Leu Ile Gly Arg His Gly Pro Gln Asn Lys
 260 265 270
 Gln Pro Phe Met Val Ala Phe Phe Lys Ala Thr Glu Val His Leu Arg
 275 280 285
 Ser Ile Arg Ser Thr Gly Gly Lys Gln Arg Ser Gln Asn Arg Ser Lys
 290 295 300
 Thr Pro Lys Asn Gln Glu Ala Leu Arg Met Ala Ser Val Ala Glu Asn
 305 310 315 320
 Ser Ser Ser Asp Gln Arg Gln Ala Cys Lys Lys His Glu Leu Tyr Val
 325 330 335
 Ser Phe Arg Asp Leu Gly Trp Gln Asp Trp Ile Ile Ala Pro Glu Gly
 340 345 350
 Tyr Ala Ala Tyr Tyr Cys Glu Gly Glu Cys Ala Phe Pro Leu Asn Ser
 355 360 365
 Tyr Met Asn Ala Thr Asn His Ala Ile Val Gln Thr Leu Val His Phe

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370                               375                               380
Ile Asn Pro Asp Thr Val Pro Lys Pro Cys Cys Ala Pro Thr Gln Leu
385                               390                               395                               400
Asn Ala Ile Ser Val Leu Tyr Phe Asp Asp Ser Ser Asn Val Ile Leu
405                               410                               415
Lys Lys Tyr Arg Asn Met Val Val Arg Ala Cys Gly Cys His
420                               425                               430

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(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1723 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Homo sapiens
- (F) TISSUE TYPE: HIPPOCAMPUS

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 490..1696
- (D) OTHER INFORMATION: /function= "OSTEOGENIC PROTEIN"
/product= "hOP2-PP"
/note= "hOP2 (cDNA)"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

```

GGCGCCGGCA GAGCAGGAGT GGCTGGAGGA GCTGTGGTTG GAGCAGGAGG TGGCACGGCA      60
GGGCTGGAGG GCTCCCTATG AGTGGCGGAG ACGGCCAGG AGGCGCTGGA GCAACAGCTC      120
CCACACCGCA CCAAGCGGTG GCTGCAGGAG CTCGCCATC GCCCTGCCGC TGCTCGGACC      180
GCGGCCACAG CCGGACTGGC GGGTACGGCG GCGACAGAGG CATTGGCCGA GAGTCCCACT      240
CCGCAGAGTA GCCCCGGCCT CGAGGCGGTG GCGTCCCGGT CCTCTCCGTC CAGGAGCCAG      300
GACAGGTGTC GCGCGGCGGG GCTCCAGGGA CCGCGCCTGA GGCCGGCTGC CCGCCCGTCC      360
CGCCCCGCCC CGCCGCCCGC CGCCGCCGA GCCAGCCTC CTTGCCGTCG GGGCGTCCCC      420
AGGCCCTGGG TCGGCCGCGG AGCCGATGCG CGCCGCTGA GCGCCCCAGC TGAGCGCCCC      480
CGGCCTGCC ATG ACC GCG CTC CCC GGC CCG CTC TGG CTC CTG GGC CTG      528
      Met Thr Ala Leu Pro Gly Pro Leu Trp Leu Leu Gly Leu
      1             5             10

GCG CTA TGC GCG CTG GGC GGG GGC GGC CCC GGC CTG CGA CCC CCG CCC      576
Ala Leu Cys Ala Leu Gly Gly Gly Gly Pro Gly Leu Arg Pro Pro Pro
      15             20             25

GGC TGT CCC CAG CGA CGT CTG GGC GCG CGC GAG CGC CGG GAC GTG CAG      624
Gly Cys Pro Gln Arg Arg Leu Gly Ala Arg Glu Arg Arg Asp Val Gln
      30             35             40             45

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CGC GAG ATC CTG GCG GTG CTC GGG CTG CCT GGG CGG CCC CGG CCC CGC Arg Glu Ile Leu Ala Val Leu Gly Leu Pro Gly Arg Pro Arg Pro Arg	672
50 55 60	
GCG CCA CCC GCC GCC TCC CGG CTG CCC GCG TCC GCG CCG CTC TTC ATG Ala Pro Pro Ala Ala Ser Arg Leu Pro Ala Ser Ala Pro Leu Phe Met	720
65 70 75	
CTG GAC CTG TAC CAC GCC ATG GCC GGC GAC GAC GAC GAG GAC GGC GCG Leu Asp Leu Tyr His Ala Met Ala Gly Asp Asp Asp Glu Asp Gly Ala	768
80 85 90	
CCC GCG GAG CGG CGC CTG GGC CGC GCC GAC CTG GTC ATG AGC TTC GTT Pro Ala Glu Arg Arg Leu Gly Arg Ala Asp Leu Val Met Ser Phe Val	816
95 100 105	
AAC ATG GTG GAG CGA GAC CGT GCC CTG GGC CAC CAG GAG CCC CAT TGG Asn Met Val Glu Arg Asp Arg Ala Leu Gly His Gln Glu Pro His Trp	864
110 115 120 125	
AAG GAG TTC CGC TTT GAC CTG ACC CAG ATC CCG GCT GGG GAG GCG GTC Lys Glu Phe Arg Phe Asp Leu Thr Gln Ile Pro Ala Gly Glu Ala Val	912
130 135 140	
ACA GCT GCG GAG TTC CGG ATT TAC AAG GTG CCC AGC ATC CAC CTG CTC Thr Ala Ala Glu Phe Arg Ile Tyr Lys Val Pro Ser Ile His Leu Leu	960
145 150 155	
AAC AGG ACC CTC CAC GTC AGC ATG TTC CAG GTG GTC CAG GAG CAG TCC Asn Arg Thr Leu His Val Ser Met Phe Gln Val Val Gln Glu Gln Ser	1008
160 165 170	
AAC AGG GAG TCT GAC TTG TTC TTT TTG GAT CTT CAG ACG CTC CGA GCT Asn Arg Glu Ser Asp Leu Phe Phe Leu Asp Leu Gln Thr Leu Arg Ala	1056
175 180 185	
GGA GAC GAG GGC TGG CTG GTG CTG GAT GTC ACA GCA GCC AGT GAC TGC Gly Asp Glu Gly Trp Leu Val Leu Asp Val Thr Ala Ala Ser Asp Cys	1104
190 195 200 205	
TGG TTG CTG AAG CGT CAC AAG GAC CTG GGA CTC CGC CTC TAT GTG GAG Trp Leu Leu Lys Arg His Lys Asp Leu Gly Leu Arg Leu Tyr Val Glu	1152
210 215 220	
ACT GAG GAC GGG CAC AGC GTG GAT CCT GGC CTG GCC GGC CTG CTG GGT Thr Glu Asp Gly His Ser Val Asp Pro Gly Leu Ala Gly Leu Leu Gly	1200
225 230 235	
CAA CGG GCC CCA CGC TCC CAA CAG CCT TTC GTG GTC ACT TTC TTC AGG Gln Arg Ala Pro Arg Ser Gln Gln Pro Phe Val Val Thr Phe Phe Arg	1248
240 245 250	
GCC AGT CCG AGT CCC ATC CGC ACC CCT CGG GCA GTG AGG CCA CTG AGG Ala Ser Pro Ser Pro Ile Arg Thr Pro Arg Ala Val Arg Pro Leu Arg	1296
255 260 265	
AGG AGG CAG CCG AAG AAA AGC AAC GAG CTG CCG CAG GCC AAC CGA CTC Arg Arg Gln Pro Lys Lys Ser Asn Glu Leu Pro Gln Ala Asn Arg Leu	1344
270 275 280 285	
CCA GGG ATC TTT GAT GAC GTC CAC GGC TCC CAC GGC CGG CAG GTC TGC Pro Gly Ile Phe Asp Asp Val His Gly Ser His Gly Arg Gln Val Cys	1392
290 295 300	

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CGT CGG CAC GAG CTC TAC GTC AGC TTC CAG GAC CTC GGC TGG CTG GAC Arg Arg His Glu Leu Tyr Val Ser Phe Gln Asp Leu Gly Trp Leu Asp 305 310 315	1440
TGG GTC ATC GCT CCC CAA GGC TAC TCG GCC TAT TAC TGT GAG GGG GAG Trp Val Ile Ala Pro Gln Gly Tyr Ser Ala Tyr Tyr Cys Glu Gly Glu 320 325 330	1488
TGC TCC TTC CCA CTG GAC TCC TGC ATG AAT GCC ACC AAC CAC GCC ATC Cys Ser Phe Pro Leu Asp Ser Cys Met Asn Ala Thr Asn His Ala Ile 335 340 345	1536
CTG CAG TCC CTG GTG CAC CTG ATG AAG CCA AAC GCA GTC CCC AAG GCG Leu Gln Ser Leu Val His Leu Met Lys Pro Asn Ala Val Pro Lys Ala 350 355 360 365	1584
TGC TGT GCA CCC ACC AAG CTG AGC GCC ACC TCT GTG CTC TAC TAT GAC Cys Cys Ala Pro Thr Lys Leu Ser Ala Thr Ser Val Leu Tyr Tyr Asp 370 375 380	1632
AGC AGC AAC AAC GTC ATC CTG CGC AAA CAC CGC AAC ATG GTG GTC AAG Ser Ser Asn Asn Val Ile Leu Arg Lys His Arg Asn Met Val Val Lys 385 390 395	1680
GCC TGC GGC TGC CAC T GAGTCAGCCC GCCCAGCCCT ACTGCAG Ala Cys Gly Cys His 400	1723

(2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 402 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

Met Thr Ala Leu Pro Gly Pro Leu Trp Leu Leu Gly Leu Ala Leu Cys 1 5 10 15
Ala Leu Gly Gly Gly Pro Gly Leu Arg Pro Pro Pro Gly Cys Pro 20 25 30
Gln Arg Arg Leu Gly Ala Arg Glu Arg Arg Asp Val Gln Arg Glu Ile 35 40 45
Leu Ala Val Leu Gly Leu Pro Gly Arg Pro Arg Pro Arg Ala Pro Pro 50 55 60
Ala Ala Ser Arg Leu Pro Ala Ser Ala Pro Leu Phe Met Leu Asp Leu 65 70 75 80
Tyr His Ala Met Ala Gly Asp Asp Asp Glu Asp Gly Ala Pro Ala Glu 85 90 95
Arg Arg Leu Gly Arg Ala Asp Leu Val Met Ser Phe Val Asn Met Val 100 105 110
Glu Arg Asp Arg Ala Leu Gly His Gln Glu Pro His Trp Lys Glu Phe

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115	120	125
Arg Phe Asp Leu Thr Gln Ile Pro Ala Gly Glu Ala Val Thr Ala Ala		
130	135	140
Glu Phe Arg Ile Tyr Lys Val Pro Ser Ile His Leu Leu Asn Arg Thr		
145	150	155
Leu His Val Ser Met Phe Gln Val Val Gln Glu Gln Ser Asn Arg Glu		
	165	170
Ser Asp Leu Phe Phe Leu Asp Leu Gln Thr Leu Arg Ala Gly Asp Glu		
	180	185
Gly Trp Leu Val Leu Asp Val Thr Ala Ala Ser Asp Cys Trp Leu Leu		
	195	200
Lys Arg His Lys Asp Leu Gly Leu Arg Leu Tyr Val Glu Thr Glu Asp		
	210	215
Gly His Ser Val Asp Pro Gly Leu Ala Gly Leu Leu Gly Gln Arg Ala		
	225	230
Pro Arg Ser Gln Gln Pro Phe Val Val Thr Phe Phe Arg Ala Ser Pro		
	245	250
Ser Pro Ile Arg Thr Pro Arg Ala Val Arg Pro Leu Arg Arg Arg Gln		
	260	265
Pro Lys Lys Ser Asn Glu Leu Pro Gln Ala Asn Arg Leu Pro Gly Ile		
	275	280
Phe Asp Asp Val His Gly Ser His Gly Arg Gln Val Cys Arg Arg His		
	290	295
Glu Leu Tyr Val Ser Phe Gln Asp Leu Gly Trp Leu Asp Trp Val Ile		
	305	310
Ala Pro Gln Gly Tyr Ser Ala Tyr Tyr Cys Glu Gly Glu Cys Ser Phe		
	325	330
Pro Leu Asp Ser Cys Met Asn Ala Thr Asn His Ala Ile Leu Gln Ser		
	340	345
Leu Val His Leu Met Lys Pro Asn Ala Val Pro Lys Ala Cys Cys Ala		
	355	360
Pro Thr Lys Leu Ser Ala Thr Ser Val Leu Tyr Tyr Asp Ser Ser Asn		
	370	375
Asn Val Ile Leu Arg Lys His Arg Asn Met Val Val Lys Ala Cys Gly		
	385	390
		395
		400
Cys His		

(2) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1926 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

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(vi) ORIGINAL SOURCE:

(A) ORGANISM: MURIDAE
(F) TISSUE TYPE: EMBRYO

(ix) FEATURE:

(A) NAME/KEY: CDS
(B) LOCATION: 93..1289
(D) OTHER INFORMATION: /function= "OSTEOGENIC PROTEIN"
/product= "mOP2-pp"
/note= "mOP2 cDNA"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

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GCCAGGCACA GGTGCGCCGT CTGGTCCTCC CCGTCTGGCG TCAGCCGAGC CCGACCAGCT      60
ACCACTGGAT GCGCGCCGGC TGAAAGTCCG AG ATG GCT ATG CGT CCC GGG CCA      113
                               Met Ala Met Arg Pro Gly Pro
                               1           5

CTC TGG CTA TTG GGC CTT GCT CTG TGC GCG CTG GGA GGC GGC CAC GGT      161
Leu Trp Leu Leu Gly Leu Ala Leu Cys Ala Leu Gly Gly Gly His Gly
          10           15           20

CCG CGT CCC CCG CAC ACC TGT CCC CAG CGT CGC CTG GGA GCG CGC GAG      209
Pro Arg Pro Pro His Thr Cys Pro Gln Arg Arg Leu Gly Ala Arg Glu
          25           30           35

CGC CGC GAC ATG CAG CGT GAA ATC CTG GCG GTG CTC GGG CTA CCG GGA      257
Arg Arg Asp Met Gln Arg Glu Ile Leu Ala Val Leu Gly Leu Pro Gly
          40           45           50           55

CGG CCC CGA CCC CGT GCA CAA CCC GCC GCT GCC CGG CAG CCA GCG TCC      305
Arg Pro Arg Pro Arg Ala Gln Pro Ala Ala Ala Arg Gln Pro Ala Ser
          60           65           70

GCG CCC CTC TTC ATG TTG GAC CTA TAC CAC GCC ATG ACC GAT GAC GAC      353
Ala Pro Leu Phe Met Leu Asp Leu Tyr His Ala Met Thr Asp Asp Asp
          75           80           85

GAC GGC GGG CCA CCA CAG GCT CAC TTA GGC CGT GCC GAC CTG GTC ATG      401
Asp Gly Gly Pro Pro Gln Ala His Leu Gly Arg Ala Asp Leu Val Met
          90           95           100

AGC TTC GTC AAC ATG GTG GAA CGC GAC CGT ACC CTG GGC TAC CAG GAG      449
Ser Phe Val Asn Met Val Glu Arg Asp Arg Thr Leu Gly Tyr Gln Glu
          105           110           115

CCA CAC TGG AAG GAA TTC CAC TTT GAC CTA ACC CAG ATC CCT GCT GGG      497
Pro His Trp Lys Glu Phe His Phe Asp Leu Thr Gln Ile Pro Ala Gly
          120           125           130           135

GAG GCT GTC ACA GCT GCT GAG TTC CGG ATC TAC AAA GAA CCC AGC ACC      545
Glu Ala Val Thr Ala Ala Glu Phe Arg Ile Tyr Lys Glu Pro Ser Thr
          140           145           150

CAC CCG CTC AAC ACA ACC CTC CAC ATC AGC ATG TTC GAA GTG GTC CAA      593
His Pro Leu Asn Thr Thr Leu His Ile Ser Met Phe Glu Val Val Gln
          155           160           165

GAG CAC TCC AAC AGG GAG TCT GAC TTG TTC TTT TTG GAT CTT CAG ACG      641
Glu His Ser Asn Arg Glu Ser Asp Leu Phe Phe Leu Asp Leu Gln Thr

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170	175	180	
CTC CGA TCT GGG GAC GAG GGC TGG CTG GTG CTG GAC ATC ACA GCA GCC Leu Arg Ser Gly Asp Glu Gly Trp Leu Val Leu Asp Ile Thr Ala Ala 185 190 195			689
AGT GAC CGA TGG CTG CTG AAC CAT CAC AAG GAC CTG GGA CTC CGC CTC Ser Asp Arg Trp Leu Leu Asn His His Lys Asp Leu Gly Leu Arg Leu 200 205 210 215			737
TAT GTG GAA ACC GCG GAT GGG CAC AGC ATG GAT CCT GGC CTG GCT GGT Tyr Val Glu Thr Ala Asp Gly His Ser Met Asp Pro Gly Leu Ala Gly 220 225 230			785
CTG CTT GGA CGA CAA GCA CCA CGC TCC AGA CAG CCT TTC ATG GTA ACC Leu Leu Gly Arg Gln Ala Pro Arg Ser Arg Gln Pro Phe Met Val Thr 235 240 245			833
TTC TTC AGG GCC AGC CAG AGT CCT GTG CGG GCC CCT CGG GCA GCG AGA Phe Phe Arg Ala Ser Gln Ser Pro Val Arg Ala Pro Arg Ala Ala Arg 250 255 260			881
CCA CTG AAG AGG AGG CAG CCA AAG AAA ACG AAC GAG CTT CCG CAC CCC Pro Leu Lys Arg Arg Gln Pro Lys Lys Thr Asn Glu Leu Pro His Pro 265 270 275			929
AAC AAA CTC CCA GGG ATC TTT GAT GAT GGC CAC GGT TCC CGC GGC AGA Asn Lys Leu Pro Gly Ile Phe Asp Asp Gly His Gly Ser Arg Gly Arg 280 285 290 295			977
GAG GTT TGC CGC AGG CAT GAG CTC TAC GTC AGC TTC CGT GAC CTT GGC Glu Val Cys Arg Arg His Glu Leu Tyr Val Ser Phe Arg Asp Leu Gly 300 305 310			1025
TGG CTG GAC TGG GTC ATC GCC CCC CAG GGC TAC TCT GCC TAT TAC TGT Trp Leu Asp Trp Val Ile Ala Pro Gln Gly Tyr Ser Ala Tyr Tyr Cys 315 320 325			1073
GAG GGG GAG TGT GCT TTC CCA CTG GAC TCC TGT ATG AAC GCC ACC AAC Glu Gly Glu Cys Ala Phe Pro Leu Asp Ser Cys Met Asn Ala Thr Asn 330 335 340			1121
CAT GCC ATC TTG CAG TCT CTG GTG CAC CTG ATG AAG CCA GAT GTT GTC His Ala Ile Leu Gln Ser Leu Val His Leu Met Lys Pro Asp Val Val 345 350 355			1169
CCC AAG GCA TGC TGT GCA CCC ACC AAA CTG AGT GCC ACC TCT GTG CTG Pro Lys Ala Cys Cys Ala Pro Thr Lys Leu Ser Ala Thr Ser Val Leu 360 365 370 375			1217
TAC TAT GAC AGC AGC AAC AAT GTC ATC CTG CGT AAA CAC CGT AAC ATG Tyr Tyr Asp Ser Ser Asn Asn Val Ile Leu Arg Lys His Arg Asn Met 380 385 390			1265
GTG GTC AAG GCC TGT GGC TGC CAC TGAGGCCCCG CCCAGCATCC TGCTTCTACT Val Val Lys Ala Cys Gly Cys His 395			1319
ACCTTACCAT CTGGCCGGGC CCCTCTCCAG AGGCAGAAAC CCTTCTATGT TATCATAGCT			1379
CAGACAGGGG CAATGGGAGG CCCTTCACCT CCCCTGGCCA CTTCTGTGTA AAATTCTGGT			1439
CTTTCCCAGT TCCTCTGTCC TTCATGGGGT TTCGGGGCTA TCACCCCGCC CTCTCCATCC			1499

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TCCTACCCCA AGCATAGACT GAATGCACAC AGCATCCCAG AGCTATGCTA ACTGAGAGGT 1559
 CTGGGGTCAG CACTGAAGGC CCACATGAGG AAGACTGATC CTTGGCCATC CTCAGCCCAC 1619
 AATGGCAAAT TCTGGATGGT CTAAGAAGGC CCTGGAATTC TAAACTAGAT GATCTGGGCT 1679
 CTCTGCACCA TTCATTGTGG CAGTTGGGAC ATTTTGTAGGT ATAACAGACA CATACTTA 1739
 GATCAATGCA TCGCTGTACT CCTTGAAATC AGAGCTAGCT TGTTAGAAAA AGAATCAGAG 1799
 CCAGGTATAG CCGTGCATGT CATTAAATCCC AGCGCTAAAG AGACAGAGAC AGGAGAATCT 1859
 CTGTGAGTTC AAGGCCACAT AGAAAGAGCC TGTCTCGGGA GCAGGAAAAA AAAAAAAAAAC 1919
 GGAATTC 1926

(2) INFORMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 399 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

Met Ala Met Arg Pro Gly Pro Leu Trp Leu Leu Gly Leu Ala Leu Cys
 1 5 10 15
 Ala Leu Gly Gly Gly His Gly Pro Arg Pro Pro His Thr Cys Pro Gln
 20 25 30
 Arg Arg Leu Gly Ala Arg Glu Arg Arg Asp Met Gln Arg Glu Ile Leu
 35 40 45
 Ala Val Leu Gly Leu Pro Gly Arg Pro Arg Pro Arg Ala Gln Pro Ala
 50 55 60
 Ala Ala Arg Gln Pro Ala Ser Ala Pro Leu Phe Met Leu Asp Leu Tyr
 65 70 75 80
 His Ala Met Thr Asp Asp Asp Asp Gly Gly Pro Pro Gln Ala His Leu
 85 90 95
 Gly Arg Ala Asp Leu Val Met Ser Phe Val Asn Met Val Glu Arg Asp
 100 105 110
 Arg Thr Leu Gly Tyr Gln Glu Pro His Trp Lys Glu Phe His Phe Asp
 115 120 125
 Leu Thr Gln Ile Pro Ala Gly Glu Ala Val Thr Ala Ala Glu Phe Arg
 130 135 140
 Ile Tyr Lys Glu Pro Ser Thr His Pro Leu Asn Thr Thr Leu His Ile
 145 150 155 160
 Ser Met Phe Glu Val Val Gln Glu His Ser Asn Arg Glu Ser Asp Leu
 165 170 175
 Phe Phe Leu Asp Leu Gln Thr Leu Arg Ser Gly Asp Glu Gly Trp Leu

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      180              185              190
Val Leu Asp Ile Thr Ala Ala Ser Asp Arg Trp Leu Leu Asn His His
  195              200              205
Lys Asp Leu Gly Leu Arg Leu Tyr Val Glu Thr Ala Asp Gly His Ser
  210              215              220
Met Asp Pro Gly Leu Ala Gly Leu Leu Gly Arg Gln Ala Pro Arg Ser
  225              230              235
Arg Gln Pro Phe Met Val Thr Phe Phe Arg Ala Ser Gln Ser Pro Val
      245              250              255
Arg Ala Pro Arg Ala Ala Arg Pro Leu Lys Arg Arg Gln Pro Lys Lys
      260              265              270
Thr Asn Glu Leu Pro His Pro Asn Lys Leu Pro Gly Ile Phe Asp Asp
      275              280              285
Gly His Gly Ser Arg Gly Arg Glu Val Cys Arg Arg His Glu Leu Tyr
      290              295              300
Val Ser Phe Arg Asp Leu Gly Trp Leu Asp Trp Val Ile Ala Pro Gln
      305              310              315
Gly Tyr Ser Ala Tyr Tyr Cys Glu Gly Glu Cys Ala Phe Pro Leu Asp
      325              330              335
Ser Cys Met Asn Ala Thr Asn His Ala Ile Leu Gln Ser Leu Val His
      340              345              350
Leu Met Lys Pro Asp Val Val Pro Lys Ala Cys Cys Ala Pro Thr Lys
      355              360              365
Leu Ser Ala Thr Ser Val Leu Tyr Tyr Asp Ser Ser Asn Asn Val Ile
      370              375              380
Leu Arg Lys His Arg Asn Met Val Val Lys Ala Cys Gly Cys His
      385              390              395

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(2) INFORMATION FOR SEQ ID NO:23:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 1368 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 1..1368
- (D) OTHER INFORMATION: /label= "60A"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

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ATG TCG GGA CTG CGA AAC ACC TCG GAG GCC GTT GCA GTG CTC GCC TCC
Met Ser Gly Leu Arg Asn Thr Ser Glu Ala Val Ala Val Leu Ala Ser
  1              5              10              15

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CTG GGA CTC GGA ATG GTT CTG CTC ATG TTC GTG GCG ACC ACG CCG CCG Leu Gly Leu Gly Met Val Leu Leu Met Phe Val Ala Thr Thr Pro Pro	96
20 25 30	
GCC GTT GAG GCC ACC CAG TCG GGG ATT TAC ATA GAC AAC GGC AAG GAC Ala Val Glu Ala Thr Gln Ser Gly Ile Tyr Ile Asp Asn Gly Lys Asp	144
35 40 45	
CAG ACG ATC ATG CAC AGA GTG CTG AGC GAG GAC GAC AAG CTG GAC GTC Gln Thr Ile Met His Arg Val Leu Ser Glu Asp Asp Lys Leu Asp Val	192
50 55 60	
TCG TAC GAG ATC CTC GAG TTC CTG GGC ATC GCC GAA CGG CCG ACG CAC Ser Tyr Glu Ile Leu Glu Phe Leu Gly Ile Ala Glu Arg Pro Thr His	240
65 70 75 80	
CTG AGC AGC CAC CAG TTG TCG CTG AGG AAG TCG GCT CCC AAG TTC CTG Leu Ser Ser His Gln Leu Ser Leu Arg Lys Ser Ala Pro Lys Phe Leu	288
85 90 95	
CTG GAC GTC TAC CAC CGC ATC ACG GCG GAG GAG GGT CTC AGC GAT CAG Leu Asp Val Tyr His Arg Ile Thr Ala Glu Glu Gly Leu Ser Asp Gln	336
100 105 110	
GAT GAG GAC GAC GAC TAC GAA CGC GGC CAT CGG TCC AGG AGG AGC GCC Asp Glu Asp Asp Asp Tyr Glu Arg Gly His Arg Ser Arg Arg Ser Ala	384
115 120 125	
GAC CTC GAG GAG GAT GAG GGC GAG CAG CAG AAG AAC TTC ATC ACC GAC Asp Leu Glu Glu Asp Glu Gly Glu Gln Gln Lys Asn Phe Ile Thr Asp	432
130 135 140	
CTG GAC AAG CGG GCC ATC GAC GAG AGC GAC ATC ATC ATG ACC TTC CTG Leu Asp Lys Arg Ala Ile Asp Glu Ser Asp Ile Ile Met Thr Phe Leu	480
145 150 155 160	
AAC AAG CGC CAC CAC AAT GTG GAC GAA CTG CGT CAC GAG CAC GGC CGT Asn Lys Arg His His Asn Val Asp Glu Leu Arg His Glu His Gly Arg	528
165 170 175	
CGC CTG TGG TTC GAC GTC TCC AAC GTG CCC AAC GAC AAC TAC CTG GTG Arg Leu Trp Phe Asp Val Ser Asn Val Pro Asn Asp Asn Tyr Leu Val	576
180 185 190	
ATG GCC GAG CTG CGC ATC TAT CAG AAC GCC AAC GAG GGC AAG TGG CTG Met Ala Glu Leu Arg Ile Tyr Gln Asn Ala Asn Glu Gly Lys Trp Leu	624
195 200 205	
ACC GCC AAC AGG GAG TTC ACC ATC ACG GTA TAC GCC ATT GGC ACC GGC Thr Ala Asn Arg Glu Phe Thr Ile Thr Val Tyr Ala Ile Gly Thr Gly	672
210 215 220	
ACG CTG GGC CAG CAC ACC ATG GAG CCG CTG TCC TCG GTG AAC ACC ACC Thr Leu Gly Gln His Thr Met Glu Pro Leu Ser Ser Val Asn Thr Thr	720
225 230 235 240	
GGG GAC TAC GTG GGC TGG TTG GAG CTC AAC GTG ACC GAG GGC CTG CAC Gly Asp Tyr Val Gly Trp Leu Glu Leu Asn Val Thr Glu Gly Leu His	768
245 250 255	
GAG TGG CTG GTC AAG TCG AAG GAC AAT CAT GGC ATC TAC ATT GGA GCA Glu Trp Leu Val Lys Ser Lys Asp Asn His Gly Ile Tyr Ile Gly Ala	816

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260	265	270	
CAC GCT GTC AAC CGA CCC GAC CGC GAG GTG AAG CTG GAC GAC ATT GGA His Ala Val Asn Arg Pro Asp Arg Glu Val Lys Leu Asp Asp Ile Gly 275 280 285			864
CTG ATC CAC CGC AAG GTG GAC GAC GAG TTC CAG CCC TTC ATG ATC GGC Leu Ile His Arg Lys Val Asp Asp Glu Phe Gln Pro Phe Met Ile Gly 290 295 300			912
TTC TTC CGC GGA CCG GAG CTG ATC AAG GCG ACG GCC CAC AGC AGC CAC Phe Phe Arg Gly Pro Glu Leu Ile Lys Ala Thr Ala His Ser Ser His 305 310 315 320			960
CAC AGG AGC AAG CGA AGC GCC AGC CAT CCA CGC AAG CGC AAG AAG TCG His Arg Ser Lys Arg Ser Ala Ser His Pro Arg Lys Arg Lys Lys Ser 325 330 335			1008
GTG TCG CCC AAC AAC GTG CCG CTG CTG GAA CCG ATG GAG AGC ACG CGC Val Ser Pro Asn Asn Val Pro Leu Leu Glu Pro Met Glu Ser Thr Arg 340 345 350			1056
AGC TGC CAG ATG CAG ACC CTG TAC ATA GAC TTC AAG GAT CTG GGC TGG Ser Cys Gln Met Gln Thr Leu Tyr Ile Asp Phe Lys Asp Leu Gly Trp 355 360 365			1104
CAT GAC TGG ATC ATC GCA CCA GAG GGC TAT GGC GCC TTC TAC TGC AGC His Asp Trp Ile Ile Ala Pro Glu Gly Tyr Gly Ala Phe Tyr Cys Ser 370 375 380			1152
GGC GAG TGC AAT TTC CCG CTC AAT GCG CAC ATG AAC GCC ACG AAC CAT Gly Glu Cys Asn Phe Pro Leu Asn Ala His Met Asn Ala Thr Asn His 385 390 395 400			1200
GCG ATC GTC CAG ACC CTG GTC CAC CTG CTG GAG CCC AAG AAG GTG CCC Ala Ile Val Gln Thr Leu Val His Leu Leu Glu Pro Lys Lys Val Pro 405 410 415			1248
AAG CCC TGC TGC GCT CCG ACC AGG CTG GGA GCA CTA CCC GTT CTG TAC Lys Pro Cys Cys Ala Pro Thr Arg Leu Gly Ala Leu Pro Val Leu Tyr 420 425 430			1296
CAC CTG AAC GAC GAG AAT GTG AAC CTG AAA AAG TAT AGA AAC ATG ATT His Leu Asn Asp Glu Asn Val Asn Leu Lys Lys Tyr Arg Asn Met Ile 435 440 445			1344
GTG AAA TCC TGC GGG TGC CAT TGA Val Lys Ser Cys Gly Cys His 450 455			1368

(2) INFORMATION FOR SEQ ID NO:24:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 455 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

Met Ser Gly Leu Arg Asn Thr Ser Glu Ala Val Ala Val Leu Ala Ser

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1	5	10	15
Leu Gly Leu Gly Met Val Leu Leu Met Phe Val Ala Thr Thr Pro Pro	20	25	30
Ala Val Glu Ala Thr Gln Ser Gly Ile Tyr Ile Asp Asn Gly Lys Asp	35	40	45
Gln Thr Ile Met His Arg Val Leu Ser Glu Asp Asp Lys Leu Asp Val	50	55	60
Ser Tyr Glu Ile Leu Glu Phe Leu Gly Ile Ala Glu Arg Pro Thr His	65	70	75
Leu Ser Ser His Gln Leu Ser Leu Arg Lys Ser Ala Pro Lys Phe Leu	85	90	95
Leu Asp Val Tyr His Arg Ile Thr Ala Glu Glu Gly Leu Ser Asp Gln	100	105	110
Asp Glu Asp Asp Asp Tyr Glu Arg Gly His Arg Ser Arg Arg Ser Ala	115	120	125
Asp Leu Glu Glu Asp Glu Gly Glu Gln Gln Lys Asn Phe Ile Thr Asp	130	135	140
Leu Asp Lys Arg Ala Ile Asp Glu Ser Asp Ile Ile Met Thr Phe Leu	145	150	155
Asn Lys Arg His His Asn Val Asp Glu Leu Arg His Glu His Gly Arg	165	170	175
Arg Leu Trp Phe Asp Val Ser Asn Val Pro Asn Asp Asn Tyr Leu Val	180	185	190
Met Ala Glu Leu Arg Ile Tyr Gln Asn Ala Asn Glu Gly Lys Trp Leu	195	200	205
Thr Ala Asn Arg Glu Phe Thr Ile Thr Val Tyr Ala Ile Gly Thr Gly	210	215	220
Thr Leu Gly Gln His Thr Met Glu Pro Leu Ser Ser Val Asn Thr Thr	225	230	235
Gly Asp Tyr Val Gly Trp Leu Glu Leu Asn Val Thr Glu Gly Leu His	245	250	255
Glu Trp Leu Val Lys Ser Lys Asp Asn His Gly Ile Tyr Ile Gly Ala	260	265	270
His Ala Val Asn Arg Pro Asp Arg Glu Val Lys Leu Asp Asp Ile Gly	275	280	285
Leu Ile His Arg Lys Val Asp Asp Glu Phe Gln Pro Phe Met Ile Gly	290	295	300
Phe Phe Arg Gly Pro Glu Leu Ile Lys Ala Thr Ala His Ser Ser His	305	310	315
His Arg Ser Lys Arg Ser Ala Ser His Pro Arg Lys Arg Lys Lys Ser	325	330	335
Val Ser Pro Asn Asn Val Pro Leu Leu Glu Pro Met Glu Ser Thr Arg			

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340	345	350
Ser Cys Gln Met Gln Thr Leu Tyr Ile Asp Phe Lys Asp Leu Gly Trp		
355	360	365
His Asp Trp Ile Ile Ala Pro Glu Gly Tyr Gly Ala Phe Tyr Cys Ser		
370	375	380
Gly Glu Cys Asn Phe Pro Leu Asn Ala His Met Asn Ala Thr Asn His		
385	390	395
Ala Ile Val Gln Thr Leu Val His Leu Leu Glu Pro Lys Lys Val Pro		
405	410	415
Lys Pro Cys Cys Ala Pro Thr Arg Leu Gly Ala Leu Pro Val Leu Tyr		
420	425	430
His Leu Asn Asp Glu Asn Val Asn Leu Lys Lys Tyr Arg Asn Met Ile		
435	440	445
Val Lys Ser Cys Gly Cys His		
450	455	

(2) INFORMATION FOR SEQ ID NO:25:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1674 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 69..1268
- (D) OTHER INFORMATION: /note= "mOP3-pp"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

GGATCCGCGG CGCTGTCCCA TCCTTGTCGT CGAGGCGTCG CTGGATGCGA GTCCGCTAAA	60
CGTCCGAG ATG GCT GCG CGT CCG GGA CTC CTA TGG CTA CTG GGC CTG GCT	110
Met Ala Ala Arg Pro Gly Leu Leu Trp Leu Leu Gly Leu Ala	
1 5 10	
CTG TGC GTG TTG GGC GGC GGT CAC CTC TCG CAT CCC CCG CAC GTC TTT	158
Leu Cys Val Leu Gly Gly Gly His Leu Ser His Pro Pro His Val Phe	
15 20 25 30	
CCC CAG CGT CGA CTA GGA GTA CGC GAG CCC CGC GAC ATG CAG CGC GAG	206
Pro Gln Arg Arg Gly Val Arg Glu Pro Arg Asp Met Gln Arg Glu	
35 40 45	
ATT CGG GAG GTG CTG GGG CTA GCC GGG CGG CCC CGA TCC CGA GCA CCG	254
Ile Arg Glu Val Leu Gly Leu Ala Gly Arg Pro Arg Ser Arg Ala Pro	
50 55 60	
GTC GGG GCT GCC CAG CAG CCA GCG TCT GCG CCC CTC TTT ATG TTG GAC	302
Val Gly Ala Ala Gln Gln Pro Ala Ser Ala Pro Leu Phe Met Leu Asp	

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65	70	75	
CTG TAC CGT GCC ATG ACG GAT GAC AGT GGC GGT GGG ACC CCG CAG CCT Leu Tyr Arg Ala Met Thr Asp Asp Ser Gly Gly Gly Thr Pro Gln Pro 80 85 90			350
CAC TTG GAC CGT GCT GAC CTG ATT ATG AGC TTT GTC AAC ATA GTG GAA His Leu Asp Arg Ala Asp Leu Ile Met Ser Phe Val Asn Ile Val Glu 95 100 105 110			398
CGC GAC CGT ACC CTG GGC TAC CAG GAG CCA CAC TGG AAG GAA TTC CAC Arg Asp Arg Thr Leu Gly Tyr Gln Glu Pro His Trp Lys Glu Phe His 115 120 125			446
TTT GAC CTA ACC CAG ATC CCT GCT GGG GAG GCT GTC ACA GCT GCT GAG Phe Asp Leu Thr Gln Ile Pro Ala Gly Glu Ala Val Thr Ala Ala Glu 130 135 140			494
TTC CGG ATC TAC AAA GAA CCC AGT ACC CAC CCG CTC AAC ACA ACC CTC Phe Arg Ile Tyr Lys Glu Pro Ser Thr His Pro Leu Asn Thr Thr Leu 145 150 155			542
CAC ATC AGC ATG TTC GAA GTG GTC CAA GAG CAC TCC AAC AGG GAG TCT His Ile Ser Met Phe Glu Val Val Gln Glu His Ser Asn Arg Glu Ser 160 165 170			590
GAC TTG TTC TTT TTG GAT CTT CAG ACG CTC CGA TCT GGG GAC GAG GGC Asp Leu Phe Phe Leu Asp Leu Gln Thr Leu Arg Ser Gly Asp Glu Gly 175 180 185 190			638
TGG CTG GTG CTG GAC ATC ACA GCA GCC AGT GAC CGA TGG CTG CTG AAC Trp Leu Val Leu Asp Ile Thr Ala Ala Ser Asp Arg Trp Leu Leu Asn 195 200 205			686
CAT CAC AAG GAC CTA GGA CTC CGC CTC TAT GTG GAA ACC GAG GAT GGG His His Lys Asp Leu Gly Leu Arg Leu Tyr Val Glu Thr Glu Asp Gly 210 215 220			734
CAC AGC ATA GAT CCT GGC CTA GCT GGT CTG CTT GGA CGA CAA GCA CCA His Ser Ile Asp Pro Gly Leu Ala Gly Leu Leu Gly Arg Gln Ala Pro 225 230 235			782
CGC TCC AGA CAG CCT TTC ATG GTT GGT TTC TTC AGG GCC AAC CAG AGT Arg Ser Arg Gln Pro Phe Met Val Gly Phe Phe Arg Ala Asn Gln Ser 240 245 250			830
CCT GTG CGG GCC CCT CGA ACA GCA AGA CCA CTG AAG AAG AAG CAG CTA Pro Val Arg Ala Pro Arg Thr Ala Arg Pro Leu Lys Lys Lys Gln Leu 255 260 265 270			878
AAT CAA ATC AAC CAG CTG CCG CAC TCC AAC AAA CAC CTA GGA ATC CTT Asn Gln Ile Asn Gln Leu Pro His Ser Asn Lys His Leu Gly Ile Leu 275 280 285			926
GAT GAT GGC CAC GGT TCT CAC GGC AGA GAA GTT TGC CGC AGG CAT GAG Asp Asp Gly His Gly Ser His Gly Arg Glu Val Cys Arg Arg His Glu 290 295 300			974
CTC TAT GTC AGC TTC CGT GAC CTT GGC TGG CTG GAC TCT GTC ATT GCC Leu Tyr Val Ser Phe Arg Asp Leu Gly Trp Leu Asp Ser Val Ile Ala 305 310 315			1022
CCC CAG GGC TAC TCC GCC TAT TAC TGT GCT GGG GAG TGC ATC TAC CCA			1070

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Pro	Gln	Gly	Tyr	Ser	Ala	Tyr	Tyr	Cys	Ala	Gly	Glu	Cys	Ile	Tyr	Pro		
320						325					330						
CTG	AAC	TCC	TGT	ATG	AAC	TCC	ACC	AAC	CAC	GCC	ACT	ATG	CAG	GCC	CTG	1118	
Leu	Asn	Ser	Cys	Met	Asn	Ser	Thr	Asn	His	Ala	Thr	Met	Gln	Ala	Leu		
335					340					345					350		
GTA	CAT	CTG	ATG	AAG	CCA	GAT	ATC	ATC	CCC	AAG	GTG	TGC	TGT	GTG	CCT	1166	
Val	His	Leu	Met	Lys	Pro	Asp	Ile	Ile	Pro	Lys	Val	Cys	Cys	Val	Pro		
				355					360					365			
ACT	GAG	CTG	AGT	GCC	ATT	TCT	CTG	CTC	TAC	TAT	GAT	AGA	AAC	AAT	AAT	1214	
Thr	Glu	Leu	Ser	Ala	Ile	Ser	Leu	Leu	Tyr	Tyr	Asp	Arg	Asn	Asn	Asn		
			370				375						380				
GTC	ATC	CTG	CGC	AGG	GAG	CGC	AAC	ATG	GTA	GTC	CAG	GCC	TGT	GGC	TGC	1262	
Val	Ile	Leu	Arg	Arg	Glu	Arg	Asn	Met	Val	Val	Gln	Ala	Cys	Gly	Cys		
		385					390					395					
CAC	TGAGTCCCTG	CCCAACAGCC	TGCTGCCATC	CCATCTATCT	AGTCAGGCCT											1315	
His																	
	400																
CTCTTCCAAG	GCAGGAAACC	AACAAAGAGG	GAAGGCAGTG	CTTTCAACTC	CATGTCCACA											1375	
TTCACAGTCT	TGGCCCTCTC	TGTTCTTTTT	GCCAAGGCTG	AGAAGATGGT	CCTAGTTATA											1435	
ACCTTGGTGA	CCTCAGTAGC	CCGATCTCTC	ATCTCCCCAA	ACTCCCCAAT	GCAGCCAGGG											1495	
GCATCTATGT	CCTTTGGGAT	TGGGCACAGA	AGTCCAATTT	ACCAACTTAT	TCATGAGTCA											1555	
CTACTGGCCC	AGCCTGGACT	TGAACCTGGA	ACACAGGGTA	GAGCTCAGGC	TCTTCAGTAT											1615	
CCATCAGAAG	ATTTAGGTGT	GTGCAGACAT	GACCACACTC	CCCCTAGCAC	TCCATAGCC											1674	

(2) INFORMATION FOR SEQ ID NO:26:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 399 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

Met	Ala	Ala	Arg	Pro	Gly	Leu	Leu	Trp	Leu	Leu	Gly	Leu	Ala	Leu	Cys		
1				5					10					15			
Val	Leu	Gly	Gly	Gly	His	Leu	Ser	His	Pro	Pro	His	Val	Phe	Pro	Gln		
		20						25					30				
Arg	Arg	Leu	Gly	Val	Arg	Glu	Pro	Arg	Asp	Met	Gln	Arg	Glu	Ile	Arg		
		35					40					45					
Glu	Val	Leu	Gly	Leu	Ala	Gly	Arg	Pro	Arg	Ser	Arg	Ala	Pro	Val	Gly		
	50					55					60						
Ala	Ala	Gln	Gln	Pro	Ala	Ser	Ala	Pro	Leu	Phe	Met	Leu	Asp	Leu	Tyr		
65				70						75				80			
Arg	Ala	Met	Thr	Asp	Asp	Ser	Gly	Gly	Gly	Thr	Pro	Gln	Pro	His	Leu		

			85						90								95
Asp	Arg	Ala	Asp	Leu	Ile	Met	Ser	Phe	Val	Asn	Ile	Val	Glu	Arg	Asp		
			100					105					110				
Arg	Thr	Leu	Gly	Tyr	Gln	Glu	Pro	His	Trp	Lys	Glu	Phe	His	Phe	Asp		
		115					120					125					
Leu	Thr	Gln	Ile	Pro	Ala	Gly	Glu	Ala	Val	Thr	Ala	Ala	Glu	Phe	Arg		
		130				135					140						
Ile	Tyr	Lys	Glu	Pro	Ser	Thr	His	Pro	Leu	Asn	Thr	Thr	Leu	His	Ile		
145					150					155					160		
Ser	Met	Phe	Glu	Val	Val	Gln	Glu	His	Ser	Asn	Arg	Glu	Ser	Asp	Leu		
				165					170					175			
Phe	Phe	Leu	Asp	Leu	Gln	Thr	Leu	Arg	Ser	Gly	Asp	Glu	Gly	Trp	Leu		
			180					185					190				
Val	Leu	Asp	Ile	Thr	Ala	Ala	Ser	Asp	Arg	Trp	Leu	Leu	Asn	His	His		
		195					200					205					
Lys	Asp	Leu	Gly	Leu	Arg	Leu	Tyr	Val	Glu	Thr	Glu	Asp	Gly	His	Ser		
	210					215					220						
Ile	Asp	Pro	Gly	Leu	Ala	Gly	Leu	Leu	Gly	Arg	Gln	Ala	Pro	Arg	Ser		
225					230					235					240		
Arg	Gln	Pro	Phe	Met	Val	Gly	Phe	Phe	Arg	Ala	Asn	Gln	Ser	Pro	Val		
				245					250					255			
Arg	Ala	Pro	Arg	Thr	Ala	Arg	Pro	Leu	Lys	Lys	Lys	Gln	Leu	Asn	Gln		
			260					265					270				
Ile	Asn	Gln	Leu	Pro	His	Ser	Asn	Lys	His	Leu	Gly	Ile	Leu	Asp	Asp		
		275					280					285					
Gly	His	Gly	Ser	His	Gly	Arg	Glu	Val	Cys	Arg	Arg	His	Glu	Leu	Tyr		
	290					295					300						
Val	Ser	Phe	Arg	Asp	Leu	Gly	Trp	Leu	Asp	Ser	Val	Ile	Ala	Pro	Gln		
305					310					315					320		
Gly	Tyr	Ser	Ala	Tyr	Tyr	Cys	Ala	Gly	Glu	Cys	Ile	Tyr	Pro	Leu	Asn		
				325					330					335			
Ser	Cys	Met	Asn	Ser	Thr	Asn	His	Ala	Thr	Met	Gln	Ala	Leu	Val	His		
			340					345					350				
Leu	Met	Lys	Pro	Asp	Ile	Ile	Pro	Lys	Val	Cys	Cys	Val	Pro	Thr	Glu		
		355					360					365					
Leu	Ser	Ala	Ile	Ser	Leu	Leu	Tyr	Tyr	Asp	Arg	Asn	Asn	Asn	Val	Ile		
						375					380						
Leu	Arg	Arg	Glu	Arg	Asn	Met	Val	Val	Gln	Ala	Cys	Gly	Cys	His			
385					390					395							

(2) INFORMATION FOR SEQ ID NO:27:

(i) SEQUENCE CHARACTERISTICS:

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- (A) LENGTH: 104 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(ix) FEATURE:

- (A) NAME/KEY: Protein
- (B) LOCATION: 1..104
- (D) OTHER INFORMATION: /note= "BMP3"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

```

Cys Ala Arg Arg Tyr Leu Lys Val Asp Phe Ala Asp Ile Gly Trp Ser
1      5      10      15
Glu Trp Ile Ile Ser Pro Lys Ser Phe Asp Ala Tyr Tyr Cys Ser Gly
20      25      30
Ala Cys Gln Phe Pro Met Pro Lys Ser Leu Lys Pro Ser Asn His Ala
35      40      45
Thr Ile Gln Ser Ile Val Ala Arg Ala Val Gly Val Val Pro Gly Ile
50      55      60
Pro Glu Pro Cys Cys Val Pro Glu Lys Met Ser Ser Leu Ser Ile Leu
65      70      75      80
Phe Phe Asp Glu Asn Lys Asn Val Val Leu Lys Val Tyr Pro Asn Met
85      90      95
Thr Val Glu Ser Cys Ala Cys Arg
100

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(2) INFORMATION FOR SEQ ID NO:28:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 102 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: HOMO SAPIENS

(ix) FEATURE:

- (A) NAME/KEY: Protein
- (B) LOCATION: 1..102
- (D) OTHER INFORMATION: /note= "BMP5"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

```

Cys Lys Lys His Glu Leu Tyr Val Ser Phe Arg Asp Leu Gly Trp Gln
1      5      10      15
Asp Trp Ile Ile Ala Pro Glu Gly Tyr Ala Ala Phe Tyr Cys Asp Gly
20      25      30

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Glu Cys Ser Phe Pro Leu Asn Ala His Met Asn Ala Thr Asn His Ala
 35 40 45

Ile Val Gln Thr Leu Val His Leu Met Phe Pro Asp His Val Pro Lys
 50 55 60

Pro Cys Cys Ala Pro Thr Lys Leu Asn Ala Ile Ser Val Leu Tyr Phe
 65 70 75 80

Asp Asp Ser Ser Asn Val Ile Leu Lys Lys Tyr Arg Asn Met Val Val
 85 90 95

Arg Ser Cys Gly Cys His
 100

(2) INFORMATION FOR SEQ ID NO:29:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 102 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: HOMO SAPIENS

(ix) FEATURE:

- (A) NAME/KEY: Protein
- (B) LOCATION: 1..102
- (D) OTHER INFORMATION: /note= "BMP6"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

Cys Arg Lys His Glu Leu Tyr Val Ser Phe Gln Asp Leu Gly Trp Gln
 1 5 10 15

Asp Trp Ile Ile Ala Pro Lys Gly Tyr Ala Ala Asn Tyr Cys Asp Gly
 20 25 30

Glu Cys Ser Phe Pro Leu Asn Ala His Met Asn Ala Thr Asn His Ala
 35 40 45

Ile Val Gln Thr Leu Val His Leu Met Asn Pro Glu Tyr Val Pro Lys
 50 55 60

Pro Cys Cys Ala Pro Thr Lys Leu Asn Ala Ile Ser Val Leu Tyr Phe
 65 70 75 80

Asp Asp Asn Ser Asn Val Ile Leu Lys Lys Tyr Arg Trp Met Val Val
 85 90 95

Arg Ala Cys Gly Cys His
 100

(2) INFORMATION FOR SEQ ID NO:30:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1247 base pairs
- (B) TYPE: nucleic acid

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(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vi) ORIGINAL SOURCE:

(A) ORGANISM: HOMO SAPIENS

(F) TISSUE TYPE: BRAIN

(ix) FEATURE:

(A) NAME/KEY: CDS

(B) LOCATION: 84..1199

(D) OTHER INFORMATION: /product= "GDF-1"
/note= "GDF-1 cDNA"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

GGGGACACCG GCCCCGCCCT CAGCCCACTG GTCCCCGGCC GCCGCGGACC CTGCGCACTC	60
TCTGGTCATC GCCTGGGAGG AAG ATG CCA CCG CCG CAG CAA GGT CCC TGC	110
Met Pro Pro Pro Gln Gln Gly Pro Cys	
1 5	
GGC CAC CAC CTC CTC CTC CTC CTG GCC CTG CTG CTG CCC TCG CTG CCC	158
Gly His His Leu Leu Leu Leu Leu Ala Leu Leu Leu Pro Ser Leu Pro	
10 15 20 25	
CTG ACC CGC GCC CCC GTG CCC CCA GGC CCA GCC GCC GCC CTG CTC CAG	206
Leu Thr Arg Ala Pro Val Pro Pro Gly Pro Ala Ala Ala Leu Leu Gln	
30 35 40	
GCT CTA GGA CTG CGC GAT GAG CCC CAG GGT GCC CCC AGG CTC CGG CCG	254
Ala Leu Gly Leu Arg Asp Glu Pro Gln Gly Ala Pro Arg Leu Arg Pro	
45 50 55	
GTT CCC CCG GTC ATG TGG CGC CTG TTT CGA CGC CGG GAC CCC CAG GAG	302
Val Pro Pro Val Met Trp Arg Leu Phe Arg Arg Arg Asp Pro Gln Glu	
60 65 70	
ACC AGG TCT GGC TCG CGG CGG ACG TCC CCA GGG GTC ACC CTG CAA CCG	350
Thr Arg Ser Gly Ser Arg Arg Thr Ser Pro Gly Val Thr Leu Gln Pro	
75 80 85	
TGC CAC GTG GAG GAG CTG GGG GTC GCC GGA AAC ATC GTG CGC CAC ATC	398
Cys His Val Glu Glu Leu Gly Val Ala Gly Asn Ile Val Arg His Ile	
90 95 100 105	
CCG GAC CGC GGT GCG CCC ACC CGG GCC TCG GAG CCT GTC TCG GCC GCG	446
Pro Asp Arg Gly Ala Pro Thr Arg Ala Ser Glu Pro Val Ser Ala Ala	
110 115 120	
GGG CAT TGC CCT GAG TGG ACA GTC GTC TTC GAC CTG TCG GCT GTG GAA	494
Gly His Cys Pro Glu Trp Thr Val Val Phe Asp Leu Ser Ala Val Glu	
125 130 135	
CCC GCT GAG CGC CCG AGC CGG GCC CGC CTG GAG CTG CGT TTC GCG GCG	542
Pro Ala Glu Arg Pro Ser Arg Ala Arg Leu Glu Leu Arg Phe Ala Ala	
140 145 150	
GCG GCG GCG GCA GCC CCG GAG GGC GGC TGG GAG CTG AGC GTG GCG CAA	590
Ala Ala Ala Ala Ala Pro Glu Gly Gly Trp Glu Leu Ser Val Ala Gln	
155 160 165	

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GCG GGC CAG GGC GCG GGC GCG GAC CCC GGG CCG GTG CTG CTC CGC CAG Ala Gly Gln Gly Ala Gly Ala Asp Pro Gly Pro Val Leu Leu Arg Gln 170 175 180 185	638
TTG GTG CCC GCC CTG GGG CCG CCA GTG CGC GCG GAG CTG CTG GGC GCC Leu Val Pro Ala Leu Gly Pro Pro Val Arg Ala Glu Leu Leu Gly Ala 190 195 200	686
GCT TGG GCT CGC AAC GCC TCA TGG CCG CGC AGC CTC CGC CTG GCG CTG Ala Trp Ala Arg Asn Ala Ser Trp Pro Arg Ser Leu Arg Leu Ala Leu 205 210 215	734
GCG CTA CGC CCC CGG GCC CCT GCC GCC TGC GCG CGC CTG GCC GAG GCC Ala Leu Arg Pro Arg Ala Pro Ala Ala Cys Ala Arg Leu Ala Glu Ala 220 225 230	782
TCG CTG CTG CTG GTG ACC CTC GAC CCG CGC CTG TGC CAC CCC CTG GCC Ser Leu Leu Leu Val Thr Leu Asp Pro Arg Leu Cys His Pro Leu Ala 235 240 245	830
CGG CCG CGG CGC GAC GCC GAA CCC GTG TTG GGC GGC GGC CCC GGG GGC Arg Pro Arg Arg Asp Ala Glu Pro Val Leu Gly Gly Gly Pro Gly Gly 250 255 260 265	878
GCT TGT CGC GCG CGG CGG CTG TAC GTG AGC TTC CGC GAG GTG GGC TGG Ala Cys Arg Ala Arg Arg Leu Tyr Val Ser Phe Arg Glu Val Gly Trp 270 275 280	926
CAC CGC TGG GTC ATC GCG CCG CGC GGC TTC CTG GCC AAC TAC TGC CAG His Arg Trp Val Ile Ala Pro Arg Gly Phe Leu Ala Asn Tyr Cys Gln 285 290 295	974
GGT CAG TGC GCG CTG CCC GTC GCG CTG TCG GGG TCC GGG GGG CCG CCG Gly Gln Cys Ala Leu Pro Val Ala Leu Ser Gly Ser Gly Gly Pro Pro 300 305 310	1022
GCG CTC AAC CAC GCT GTG CTG CGC GCG CTC ATG CAC GCG GCC GCC CCG Ala Leu Asn His Ala Val Leu Arg Ala Leu Met His Ala Ala Ala Pro 315 320 325	1070
GGA GCC GCC GAC CTG CCC TGC TGC GTG CCC GCG CGC CTG TCG CCC ATC Gly Ala Ala Asp Leu Pro Cys Cys Val Pro Ala Arg Leu Ser Pro Ile 330 335 340 345	1118
TCC GTG CTC TTC TTT GAC AAC AGC GAC AAC GTG GTG CTG CGG CAG TAT Ser Val Leu Phe Phe Asp Asn Ser Asp Asn Val Val Leu Arg Gln Tyr 350 355 360	1166
GAG GAC ATG GTG GTG GAC GAG TGC GGC TGC CGC TAACCCGGGG CGGGCAGGGA Glu Asp Met Val Val Asp Glu Cys Gly Cys Arg 365 370	1219
CCCGGGCCCA ACAATAAATG CCGCGTGG	1247

(2) INFORMATION FOR SEQ ID NO:31:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 372 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

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(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:

Met Pro Pro Pro Gln Gln Gly Pro Cys Gly His His Leu Leu Leu Leu
 1 5 10 15
 Leu Ala Leu Leu Leu Pro Ser Leu Pro Leu Thr Arg Ala Pro Val Pro
 20 25 30
 Pro Gly Pro Ala Ala Ala Leu Leu Gln Ala Leu Gly Leu Arg Asp Glu
 35 40 45
 Pro Gln Gly Ala Pro Arg Leu Arg Pro Val Pro Pro Val Met Trp Arg
 50 55 60
 Leu Phe Arg Arg Arg Asp Pro Gln Glu Thr Arg Ser Gly Ser Arg Arg
 65 70 75 80
 Thr Ser Pro Gly Val Thr Leu Gln Pro Cys His Val Glu Glu Leu Gly
 85 90 95
 Val Ala Gly Asn Ile Val Arg His Ile Pro Asp Arg Gly Ala Pro Thr
 100 105 110
 Arg Ala Ser Glu Pro Val Ser Ala Ala Gly His Cys Pro Glu Trp Thr
 115 120 125
 Val Val Phe Asp Leu Ser Ala Val Glu Pro Ala Glu Arg Pro Ser Arg
 130 135 140
 Ala Arg Leu Glu Leu Arg Phe Ala Ala Ala Ala Ala Ala Pro Glu
 145 150 155 160
 Gly Gly Trp Glu Leu Ser Val Ala Gln Ala Gly Gln Gly Ala Gly Ala
 165 170 175
 Asp Pro Gly Pro Val Leu Leu Arg Gln Leu Val Pro Ala Leu Gly Pro
 180 185 190
 Pro Val Arg Ala Glu Leu Leu Gly Ala Ala Trp Ala Arg Asn Ala Ser
 195 200 205
 Trp Pro Arg Ser Leu Arg Leu Ala Leu Ala Leu Arg Pro Arg Ala Pro
 210 215 220
 Ala Ala Cys Ala Arg Leu Ala Glu Ala Ser Leu Leu Leu Val Thr Leu
 225 230 235 240
 Asp Pro Arg Leu Cys His Pro Leu Ala Arg Pro Arg Arg Asp Ala Glu
 245 250 255
 Pro Val Leu Gly Gly Gly Pro Gly Gly Ala Cys Arg Ala Arg Arg Leu
 260 265 270
 Tyr Val Ser Phe Arg Glu Val Gly Trp His Arg Trp Val Ile Ala Pro
 275 280 285
 Arg Gly Phe Leu Ala Asn Tyr Cys Gln Gly Gln Cys Ala Leu Pro Val
 290 295 300
 Ala Leu Ser Gly Ser Gly Gly Pro Pro Ala Leu Asn His Ala Val Leu
 305 310 315 320

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Arg Ala Leu Met His Ala Ala Ala Pro Gly Ala Ala Asp Leu Pro Cys
325 330 335

Cys Val Pro Ala Arg Leu Ser Pro Ile Ser Val Leu Phe Phe Asp Asn
340 345 350

Ser Asp Asn Val Val Leu Arg Gln Tyr Glu Asp Met Val Val Asp Glu
355 360 365

Cys Gly Cys Arg
370

CLAIMS

What is claimed is:

- 1 1. A method of therapy for a mammal at risk of, or afflicted with, loss of or damage to
2 myocardium, the method comprising
3 implanting a preparation of myogenic precursor cells into said mammal at a site at risk of,
4 or afflicted with, loss of or damage to myocardium, and
5 treating said myogenic precursor cells with an amount of a morphogen sufficient to
6 promote proliferation or differentiation of said myogenic precursor cells into functional
7 myocardium.
- 1 2. A method of therapy for a mammal at risk of, or afflicted with, loss of or damage to
2 myocardium, the method comprising
3 implanting a preparation of myogenic precursor cells into said mammal at a site at risk of,
4 or afflicted with, loss of or damage to myocardium, and
5 treating said mammal with an amount of an inducer of a morphogen encoded by a gene of
6 said mammal, said amount being sufficient to promote proliferation or differentiation of said
7 myogenic precursor cells into functional myocardium.
- 1 3. A method of therapy for a mammal at risk of, or afflicted with, loss of or damage to
2 myocardium, the method comprising
3 implanting a preparation of myogenic precursor cells into said mammal at a site at risk of,
4 or afflicted with, loss of or damage to myocardium, and
5 treating said myogenic precursor cells with an amount of an agonist of a morphogen
6 receptor expressed by said myogenic precursor cells, said amount being sufficient to promote
7 proliferation or differentiation of said myogenic precursor cells into functional myocardium.
- 1 4. A method of therapy for a mammal at risk of, or afflicted with, loss of or damage to
2 myocardium, the method comprising
3 implanting a preparation of myogenic precursor cells into said mammal at a site at risk of,
4 or afflicted with, loss of or damage to myocardium, and

5 treating said myogenic precursor cells with an amount of a small molecule morphogenic
6 activator, said amount being sufficient to promote proliferation or differentiation of said myogenic
7 precursor cells into functional myocardium.

1 5. A method as in any one of claims 1-4 wherein said myogenic precursor cells are selected
2 from the group consisting of mammalian skeletal muscle satellite cells, embryonic myogenic
3 precursor cells, and a histocompatible mammalian myogenic precursor cell line.

1 6. A method as in any one of claims 1-4 wherein said myogenic precursor cells are
2 autologous skeletal muscle satellite cells.

1 7. A method as in any one of claims 1-4 wherein
2 said mammal is afflicted with a condition selected from the group consisting of myocardial
3 infarction and congestive heart failure.

1 8. A method as in any one of claims 1-4 wherein said treatment step is conducted prior to
2 said implantation step.

1 9. A method as in any one of claims 1-4 wherein said treatment step is conducted
2 simultaneous with said implantation step.

1 10. A method as in any one of claims 1-4 wherein said treatment step is conducted subsequent
2 to said implantation step.

1 11. A method as in claim 10 wherein said treatment step is at least once a week for a period of
2 at least four weeks.

1 12. A method as in claim 10 wherein said treatment step is at least once a month for a period
2 of at least one year.

1 13. A method as in claim 1 wherein said morphogen treatment step is conducted with
2 morphogen at a concentration of about 0.01-1000 ng/ml.

1 14. A method as in claim 1 wherein said morphogen treatment step is conducted with
2 morphogen at a concentration of about 0.1-100 ng/ml.

1 15. A method of promoting proliferation of myogenic precursor cells or differentiation of
2 myogenic precursor cells into functional myocardium comprising the steps of:

- 3 (a) contacting said cells with a morphogen in an amount effective to induce said
4 proliferation or differentiation; and
- 5 (b) maintaining said cells in a morphogenically permissive environment.
- 1 16. A method as in claim 1 wherein said morphogen is selected from the group consisting of a
2 pro form of a morphogen, a soluble form of a morphogen, a mature morphogen, and a C-terminal
3 fragment of a morphogen comprising at least the seven cysteine domain of said morphogen.
- 1 17. A method as in claim 1 wherein said morphogen is selected from the group consisting of
2 osteogenic proteins and bone morphogenic proteins.
- 1 18. A method as in claim 1 wherein said morphogen
2 induces a cascade of tissue-specific morphogenesis culminating in the formation of
3 functional mammalian myocardium; and
4 comprises a pair of folded polypeptides, the amino acid sequence of each of which
5 comprises a sequence having at least 70% amino acid sequence homology with the C-terminal
6 seven-cysteine domain of human OP-1, mouse OP-1, human OP-2 or mouse OP-2, residues 38-
7 139 of SEQ ID NOs. 5, 6, 7 or 8, respectively.
- 1 19. A method as in claim 1 wherein said morphogen is selected from the group consisting of
2 OP-1, CBMP-2A (BMP-2), and CBMP-2B (BMP-4).
- 1 20. A therapeutic composition for promoting the repair or regeneration of mammalian
2 myocardium comprising
3 isolated mammalian myogenic precursor cells, and
4 an amount of a morphogen sufficient to promote proliferation or differentiation of said
5 myogenic precursor cells into functional myocardium in a morphogenically permissive
6 environment.
- 1 21. A therapeutic composition for promoting the repair or regeneration of mammalian
2 myocardium comprising
3 isolated mammalian myogenic precursor cells, and

4 an amount of an inducer of a morphogen sufficient to promote proliferation or
5 differentiation of said myogenic precursor cells into functional myocardium in a morphogenically
6 permissive environment.

1 22. A therapeutic composition for promoting the repair or regeneration of mammalian
2 myocardium comprising

3 isolated mammalian myogenic precursor cells, and

4 an amount of an agonist of a morphogen receptor sufficient to promote proliferation or
5 differentiation of said myogenic precursor cells into functional myocardium in a morphogenically
6 permissive environment.

1 23. A therapeutic composition for promoting the repair or regeneration of mammalian
2 myocardium comprising

3 isolated mammalian myogenic precursor cells, and

4 an amount of a small molecule morphogenic activator sufficient to promote proliferation
5 or differentiation of said myogenic precursor cells into functional myocardium in a
6 morphogenically permissive environment.

1 24. A method of culturing mammalian myogenic precursor cells comprising
2 isolating said myogenic precursor cells, and
3 culturing said myogenic precursor cells in a medium comprising an amount of a
4 morphogen sufficient to promote proliferation or differentiation of said myogenic precursor cells
5 into functional myocardium in a morphogenically permissive environment.

1 25. A method of culturing mammalian myogenic precursor cells comprising
2 isolating said myogenic precursor cells, and
3 culturing said myogenic precursor cells in a medium comprising an amount of an inducer
4 of a morphogen sufficient to promote proliferation or differentiation of said myogenic precursor
5 cells into functional myocardium in a morphogenically permissive environment.

1 26. A method of culturing mammalian myogenic precursor cells comprising
2 isolating said myogenic precursor cells, and

3 culturing said myogenic precursor cells in a medium comprising an amount of an agonist
4 of a morphogen receptor sufficient to promote proliferation or differentiation of said myogenic
5 precursor cells into functional myocardium in a morphogenically permissive environment.

1 27. A method of culturing mammalian myogenic precursor cells comprising
2 isolating said myogenic precursor cells, and
3 culturing said myogenic precursor cells in a medium comprising an amount of a small
4 molecule morphogenic activator sufficient to promote proliferation or differentiation of said
5 myogenic precursor cells into functional myocardium in a morphogenically permissive
6 environment.

1 28. A method of inducing myogenic precursor cells, naturally competent to differentiate into
2 skeletal or smooth muscle, to differentiate into cardiomyocytes, said method comprising the steps
3 of

4 (a) contacting said myogenic precursor cells with a morphogen; and
5 (b) maintaining the product of (a) in an environment morphogenically permissive for
6 cardiomyogenesis.

1 29. A method of producing replacement cardiomyocytes in a mammal in need thereof, said
2 method comprising the step of implanting into said mammal myogenic precursor cells induced by
3 the method of claim 28.

1 30. A pharmaceutical composition comprising
2 a morphogenic agent selected from the group consisting of a morphogen, a morphogen
3 inducer, an agonist of a morphogen receptor, and a small molecule morphogenic activator; and
4 a mitogen selected from the group consisting of bFGF, IGF, PDGF, LIF, ACTH, MSH,
5 and G-CSF.

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	Cys	Lys	Lys	Lys	His	Glu	Leu	Tyr	Val
hOP-1
mOP-1	...	Arg
hOP-2	...	Arg	Arg
mOP-2	...	Arg	Arg
mOP-3	...	Arg	Arg
DPP	...	Arg	Arg	Ser
Vg1	Lys	Arg	His	His
Vgr-1	Gly
CBMP-2A	Arg	Pro
CBMP-2B	...	Arg	Arg	Ser
BMP3	...	Ala	Arg	Arg	Arg	Tyr	...	Lys	...
GDF-1	...	Arg	Ala	Arg	Arg	Arg
60A	...	Gln	Met	...	Glu	Thr
BMP5
BMP6	...	Arg
	1					5			

FIG. 1A

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hOP-1	Ser	Phe	Arg	Asp	Leu	Gly	Trp	Gln	Asp
mOP-1
hOP-2	Gln	Leu	...
mOP-2	Ser	Leu	...
mOP-3	Leu	...
DPP	Asp	...	Ser	...	Val	Asp	...
Vg1	Glu	...	Lys	...	Val	Asn
Vgr-1	Gln	...	Val
CBMP-2A	Asp	...	Ser	...	Val	Asn	...
CBMP-2B	Asp	...	Ser	...	Val	Asn	...
BMP3	Asp	...	Ala	...	Ile	Ser	Glu
GDF-1	Glu	Val	His	Arg
60A	Asp	...	Lys	His	...
BMP5
BMP6	Gln

15

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FIG. 1B

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hOP-1	Trp	Ile	Ile	Ala	Pro	Glu	Gly	Tyr	Ala
mOP-1
hOP-2	...	Val	Gln	Ser
mOP-2	...	Val	Gln	Ser
mOP-3	Ser	Val	Gln	Ser
DPP	Val	Leu	Asp
Vgl	...	Val	Gln	Met
Vgr-1	Lys
CBMP-2A	Val	Pro	His
CBMP-2B	Val	Pro	Gln
BMP3	Ser	...	Lys	Ser	Phe	Asp
GDF-1	...	Val	Arg	...	Phe	Leu
60A	Gly
BMP5
BMP6	Lys
									25
									20

FIG. 1C

hOP-1	Ala	Tyr	Tyr	Cys	Glu	Gly	Glu	Cys	Ala
mOP-1
hOP-2	Ser
mOP-2
mOP-3	Ala	Ile
DPP	His	...	Lys	...	Pro
Vgl	...	Asn	Tyr	Pro
Vgr-1	...	Asn	Asp	Ser
CBMP-2A	...	Phe	His	...	Glu	...	Pro
CBMP-2B	...	Phe	His	...	Asp	...	Pro
BMP3	Ser	...	Ala	...	Gln
GDF-1	...	Asn	Gln	...	Gln
60A	...	Phe	Ser	Asn
BMP5	...	Phe	Asp	Ser
BMP6	...	Asn	Asp	Ser
				30					35

FIG. 1D

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hOP-1	Phe	Pro	Leu	Asn	Ser	Tyr	Met	Asn	Ala
mOP-1
hOP-2	Asp	...	Cys
mOP-2	Asp	...	Cys
mOP-3	Tyr	Cys	Ser
DPP	Ala	Asp	His	Phe	...	Ser
Vgl	Tyr	Thr	Glu	Ile	Leu	...	Gly
Vgr-1	Ala	His
CBMP-2A	Ala	Asp	His	Leu	...	Ser
CBMP-2B	Ala	Asp	His	Leu	...	Ser
GDF-1	Leu	...	Val	Ala	Leu	Ser	Gly	Ser**	...
BMP3	Met	Pro	Lys	Ser	Leu	Lys	Pro
60A	Ala	His
BMP5	Ala	His	Met
BMP6	Ala	His	Met

40

FIG. 1E

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	Thr	Asn	His	Ala	Ile	Val	Gln	Thr	Leu
hOP-1
mOP-1	Leu	...	Ser	...
hOP-2	Leu	...	Ser	...
mOP-2	Thr	Met	...	Ala	...
mOP-3	Val
DPP	Leu
Vgl	Ser
Vgr-1
CBMP-2A
CBMP-2B
BMP3	Ser	Thr	Ile	...	Ser	Ile
GDF-1	Leu	Val	Leu	Arg	Ala	...
60A
BMP5
BMP6
	45					50			

FIG. 1F

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hOP-1	Val	His	Phe	Ile	Asn	Pro	Glu	Thr	Val
mOP-1	Asp
hOP-2	...	His	Leu	Met	Lys	...	Asn	Ala	...
mOP-2	...	His	Leu	Met	Lys	...	Asp	Val	...
mOP-3	Leu	Met	Lys	...	Asp	Ile	Ile
DPP	...	Asn	Asn	Asn	Gly	Lys	...
Vgl	Ser	...	Glu	Asp	Ile
Vgr-1	Val	Met	Tyr	...
CBMP-2A	...	Asn	Ser	Val	...	Ser	...	Lys	Ile
CBMP-2B	...	Asn	Ser	Val	...	Ser	...	Ser	Ile
BMP3	...	Arg	Ala**	Gly	Val	Val	Pro	Gly	Ile
GDF-1	Met	...	Ala	Ala	Ala	...	Gly	Ala	Ala
60A	Leu	Leu	Glu	...	Lys	Lys	...
BMP5	Leu	Met	Phe	...	Asp	His	...
BMP6	Leu	Met	Tyr	...
		55					60		

FIG. 1G

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	Pro	Lys	Pro	Cys	Cys	Ala	Pro	Thr	Gln
hOP-1
mOP-1	Ala	Lys
hOP-2	Ala	Lys
mOP-2	Val	Val	Glu
mOP-3	Ala	Val
DPP	Leu	Val	Lys
Vgl	Lys
Vgr-1	Ala	Val	Glu
CBMP-2A	Ala	Val	Glu
CBMP-2B	Glu	Val	Lys
BMP3	Asp	Leu	Val	...	Ala	Arg
GDF-1	Arg
60A	Lys
BMP5	Lys
BMP6	Lys
									70
									65

FIG. 1H

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hOP-1	Leu	Asn	Ala	Ile	Ser	Val	Leu	Tyr	Phe
mOP-1
hOP-2	...	Ser	...	Thr	Tyr
mOP-2	...	Ser	...	Thr	Tyr
mOP-3	...	Ser	Leu	Tyr
Vgl	Met	Ser	Pro	Met	...	Phe	Tyr
Vgr-1	Val
DPP	...	Asp	Ser	Val	Ala	Met	Leu
CBMP-2A	...	Ser	Met	Leu
CBMP-2B	...	Ser	Met	Leu
BMP3	Met	Ser	Ser	Leu	...	Ile	...	Phe	Tyr
GDF-1	...	Ser	Pro	Phe	...
60A	...	Gly	...	Leu	Pro	His
BMP5
BMP6
				75					80

FIG. 11

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hOP-1	Asp	Asp	Ser	Ser	Asn	Val	Ile	Leu	Lys
mOP-1
hOP-2	...	Ser	...	Asn	Arg
mOP-2	...	Ser	...	Asn	Arg
mOP-3	...	Arg	Asn	Asn	Arg
DPP	Asn	...	Gln	...	Thr	...	Val
Vgl	...	Asn	Asn	Asp	Val	...	Arg
Vgr-1	Asn
CBMP-2A	...	Glu	Asn	Glu	Lys	...	Val
CBMP-2B	...	Glu	Tyr	Asp	Lys	...	Val
BMP3	...	Glu	Asn	Lys	Val
GDF-1	...	Asn	...	Asp	Val	...	Arg
60A	Leu	Asn	Asp	Glu	Asn
BMP5
BMP6	Asn

85

FIG. 1J

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hOP-1	Lys	Tyr	Arg	Asn	Met	Val	Val	Arg
mOP-1
hOP-2	...	His	Lys
mOP-2	...	His	Lys
mOP-3	Arg	Glu	Gln
DPP	Asn	...	Gln	Glu	...	Thr	...	Val
Vgl	His	...	Glu	Ala	...	Asp
Vgr-1
CBMP-2A	Asn	...	Gln	Asp	Glu
CBMP-2B	Asn	...	Gln	Glu	Glu
BMP3	Val	...	Pro	Thr	...	Glu
GDF-1	Gln	...	Glu	Asp	Asp
60A	Ile	...	Lys
BMP5
BMP6	Trp
	90					95		

FIG. 1K

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hOP-1	Ala	Cys	Gly	Cys	His
mOP-1
hOP-2
mOP-2
mOP-3
DPP	Gly	Arg
Vgl	Glu	Arg
Vgr-1
CBMP-2A	Gly	Arg
CBMP-2B	Gly	Arg
BMP3	Ser	...	Ala	...	Arg
GDF-1	Glu	Arg
60A	Ser
BMP5	Ser
BMP6
			100		

**Between residues 56 and 57 of BMP3 is a Val residue;
between residues 43 and 44 of GDF-1 lies the amino acid
sequence Gly-Gly-Pro-Pro.

FIG. 1L

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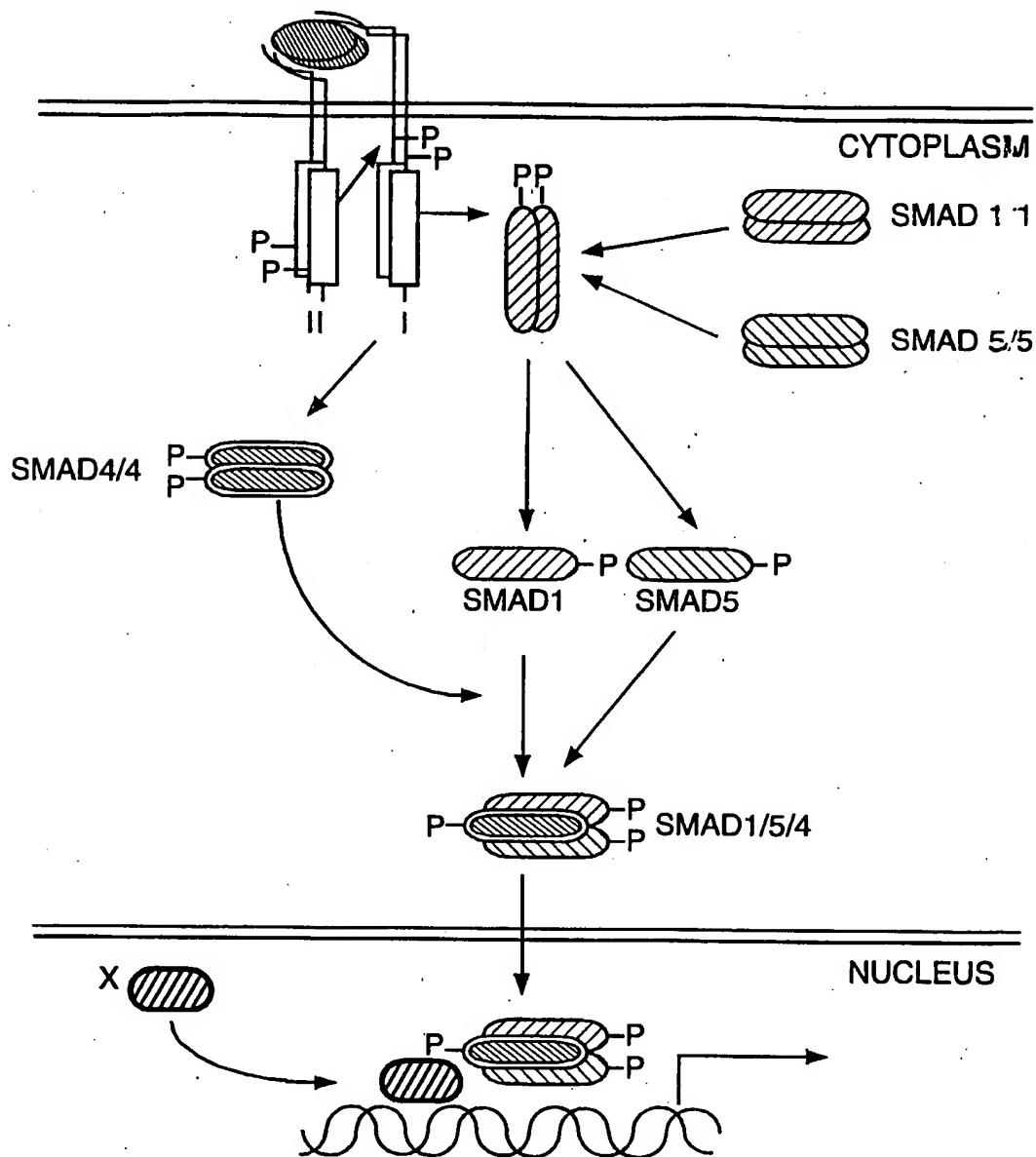


FIG. 2